San Giuseppe Moscati - Avellino
Azienda Ospedaliera di Rilievo Nazionale e di Alta Specializzazione

1° SIMPOSIO
SULLE TERAPIE INNOVATIVE
IN EMATOLOGIA

Avellino, Hotel de la Ville
30-31 Marzo 2023

I SESSIONE
Nuovi paradigmi di trattamento nella AA, EPN e mastocitosi

L'interferone nell'anemia aplastica: dal laboratorio al letto del paziente?

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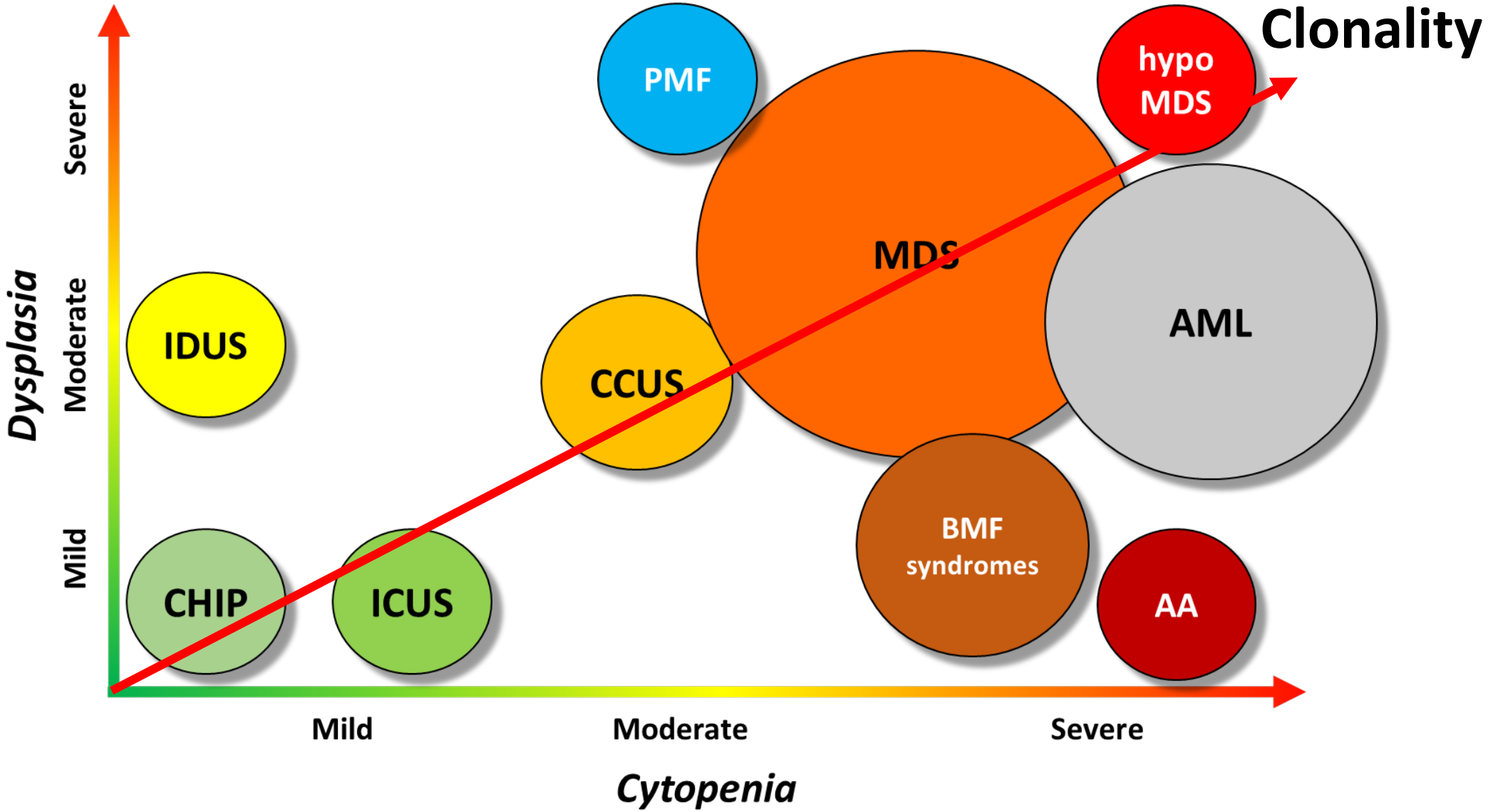
Università degli Sudi di Salerno

Hematological diseases: continuous clinical entities



CHIP, Clonal hematopoiesis of indeterminate (clinical) potential. ICUS, Idiopathic cytopenia of unknown significance. IDUS, Idiopathic dysplasia of unknown significance. CCUS, Clonal cytopenia of unknown significance. MDS, Myelodysplastic syndromes. PMF, Primary Myelofibrosis. BMF, *Bone Marrow Failure syndromes. AA, Acquired Aplastic Anemia. AML, Acute Myeloid Leukemia.*

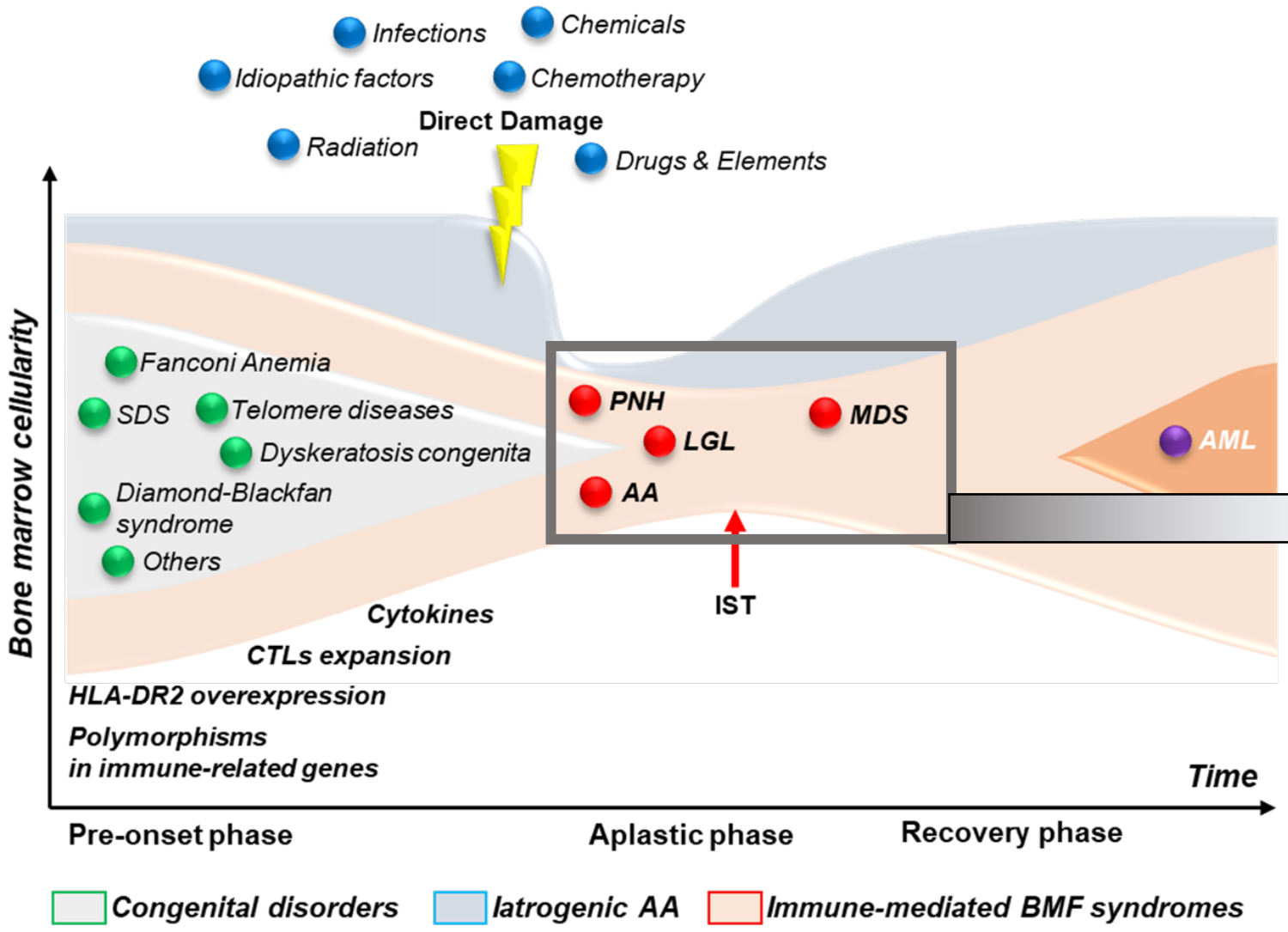
The long walk of BMF

Monet, The Japanese Footbridge

1899

1924

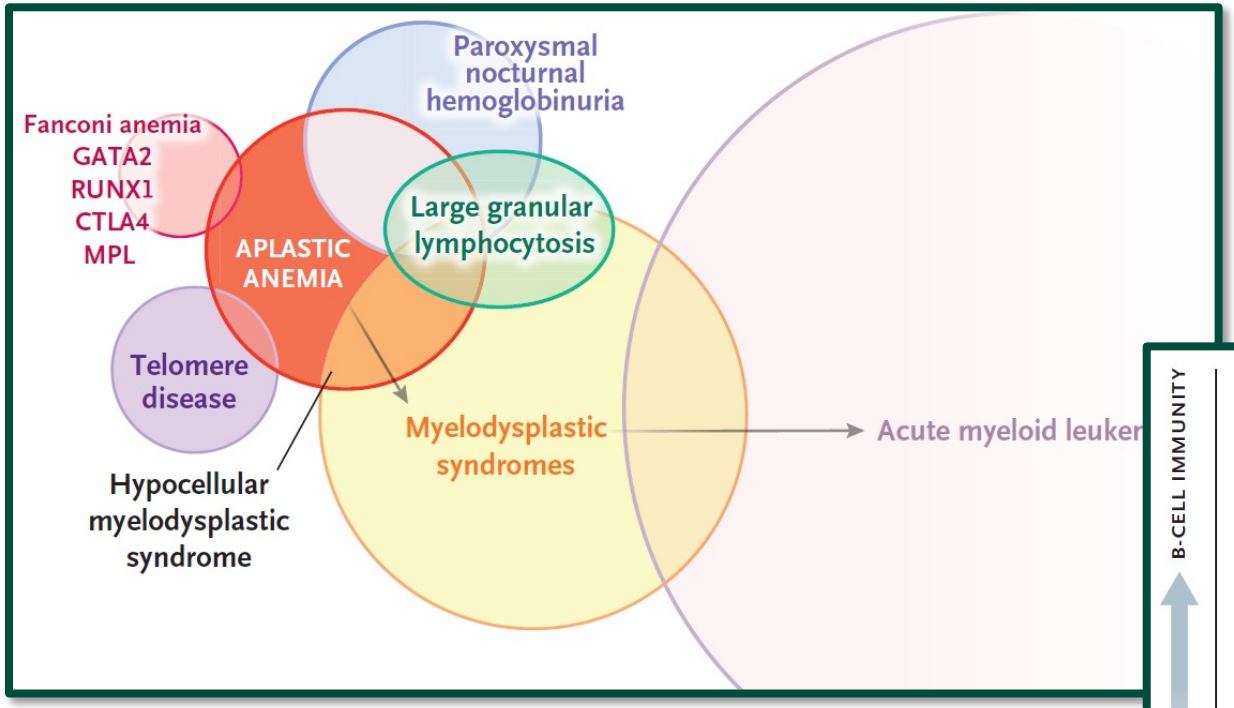
BMF – a complex classification



- Immune-mediated BMF syndromes
  - Acquired Aplastic Anemia (AA)
  - Hypoplastic myelodysplastic syndrome (hMDS)
  - Large granular lymphocyte leukemia (LGL)
  - Paroxysmal nocturnal hemoglobinuria (PNH)

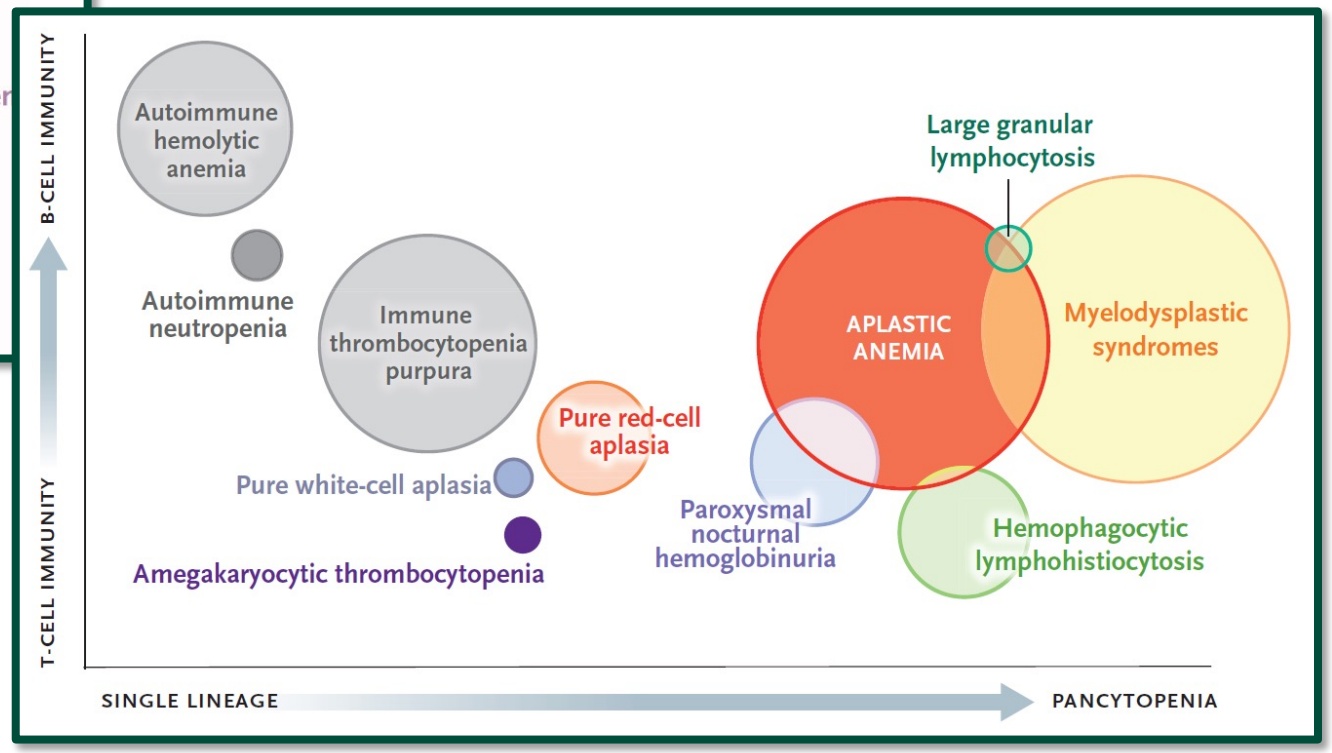
Giudice V, et al. Int J Mol Sci. 2021 Jan 12;22(2):705.

Immune-mediated BMF – many points of view



By clinical symptoms

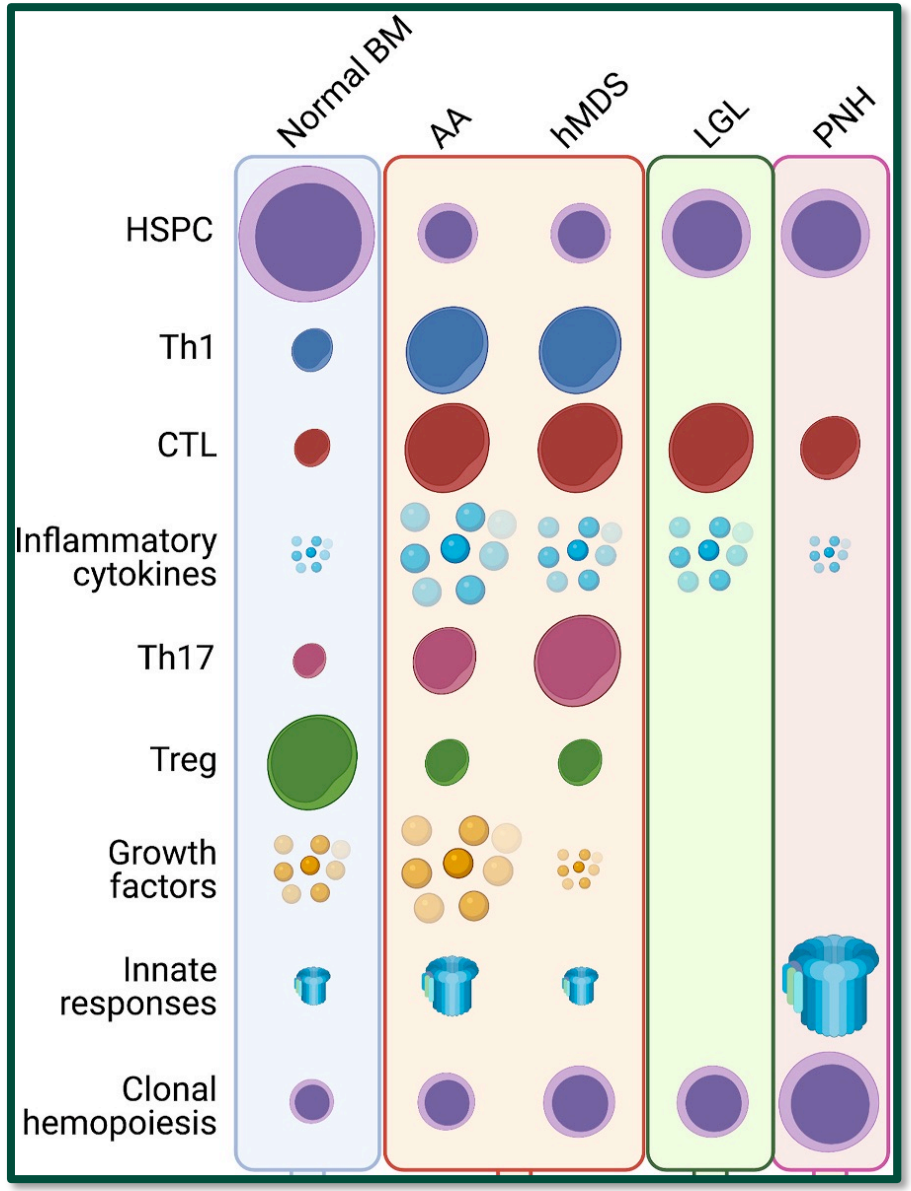
By lineage involment and type of immune response



Young NS. N Engl J Med. 2018 Oct 25;379(17):1643-1656.

Immune-mediated BMF – different responses

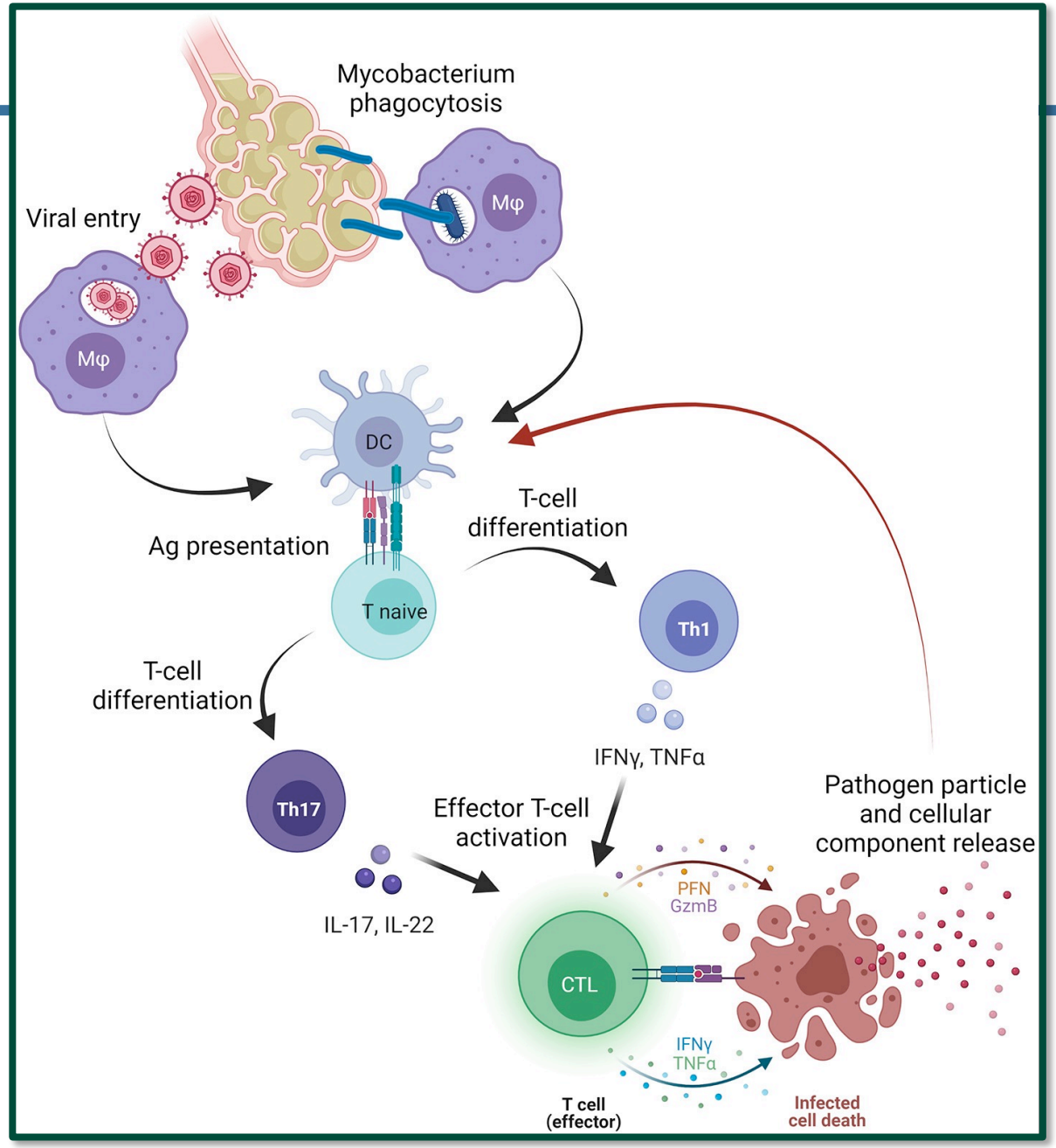
- In normal hemopoiesis, immune system is fine-tuned.
- In immune-mediated BMF, various combinations of cell subset and cytokine derangement cause a wide range of clinical manifestations.
- Mainly Th1-mediated immune responses and cytotoxic CD8+ T cell-mediated autologous immune attack against HSCs.
- Hematological recovery of blood counts after immunosuppressive therapies (ISTs) is one of the strongest evidence for the immune-mediated pathogenesis.



Patel BA, Giudice V, Young NS. Best Pract Res Clin Haematol. 2021 Jun;34(2):101276.

Back in ‘90s

- An unknown viral infection affecting stem cells might cause cross-reactivity with self-antigens and subsequent autoimmune clone expansion.
- Infected cells preferentially trigger T helper (Th) 1 response, the predominant CD4+ T cell subset involved in viral clearance through activation of cytotoxic T cells (CTLs) via interferon-γ (IFNγ) or tumor necrosis factor-α (TNF-α).
- CTLs expand and directly kill cells also through Fas-ligand (FasL) secretion.

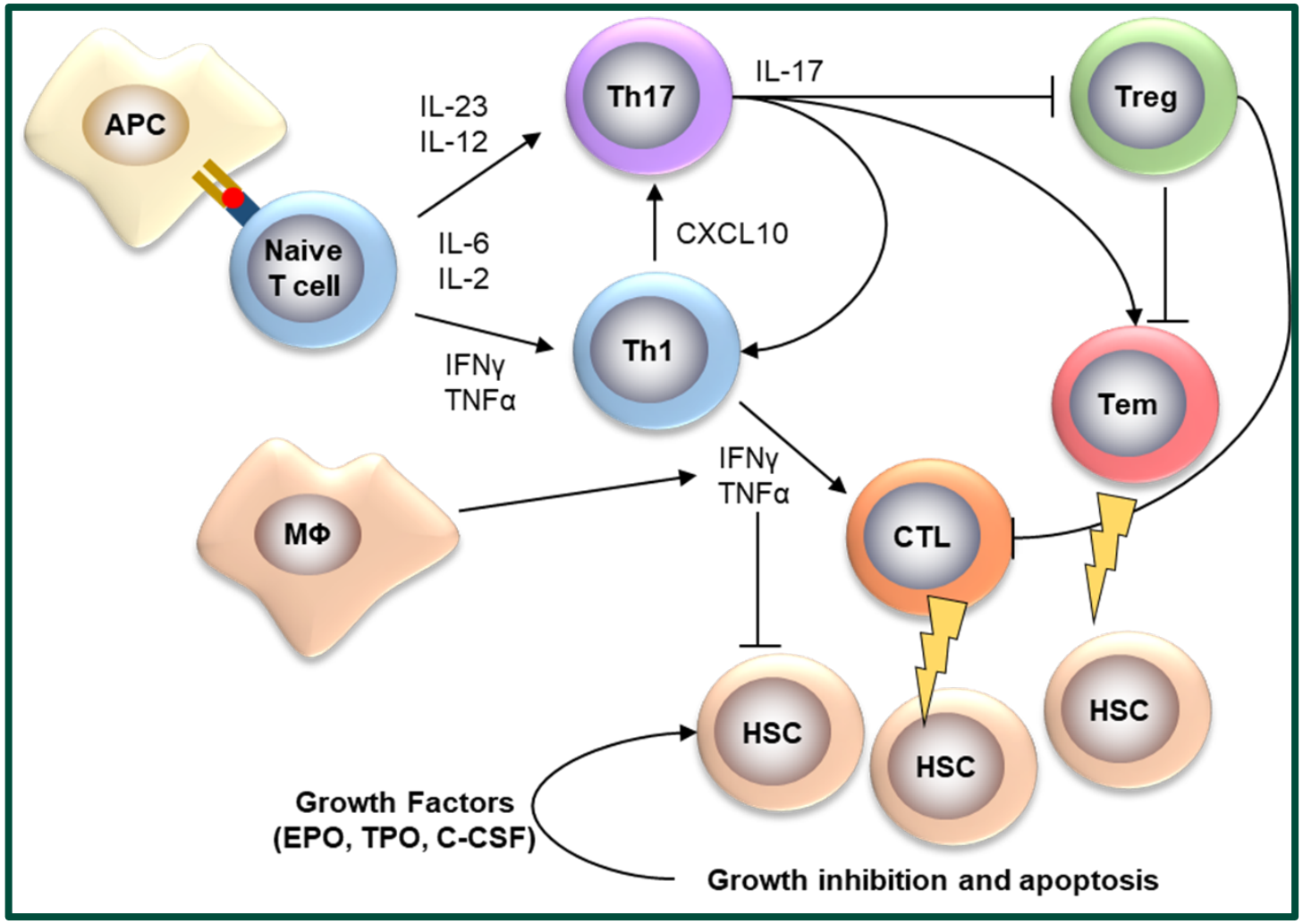


Young NS. JAMA. 1999 Jul 21;282(3):271-8.

Giudice V, Risitano AM, Selleri C. Front Med (Lausanne). 2021 Nov 4;8:757730.

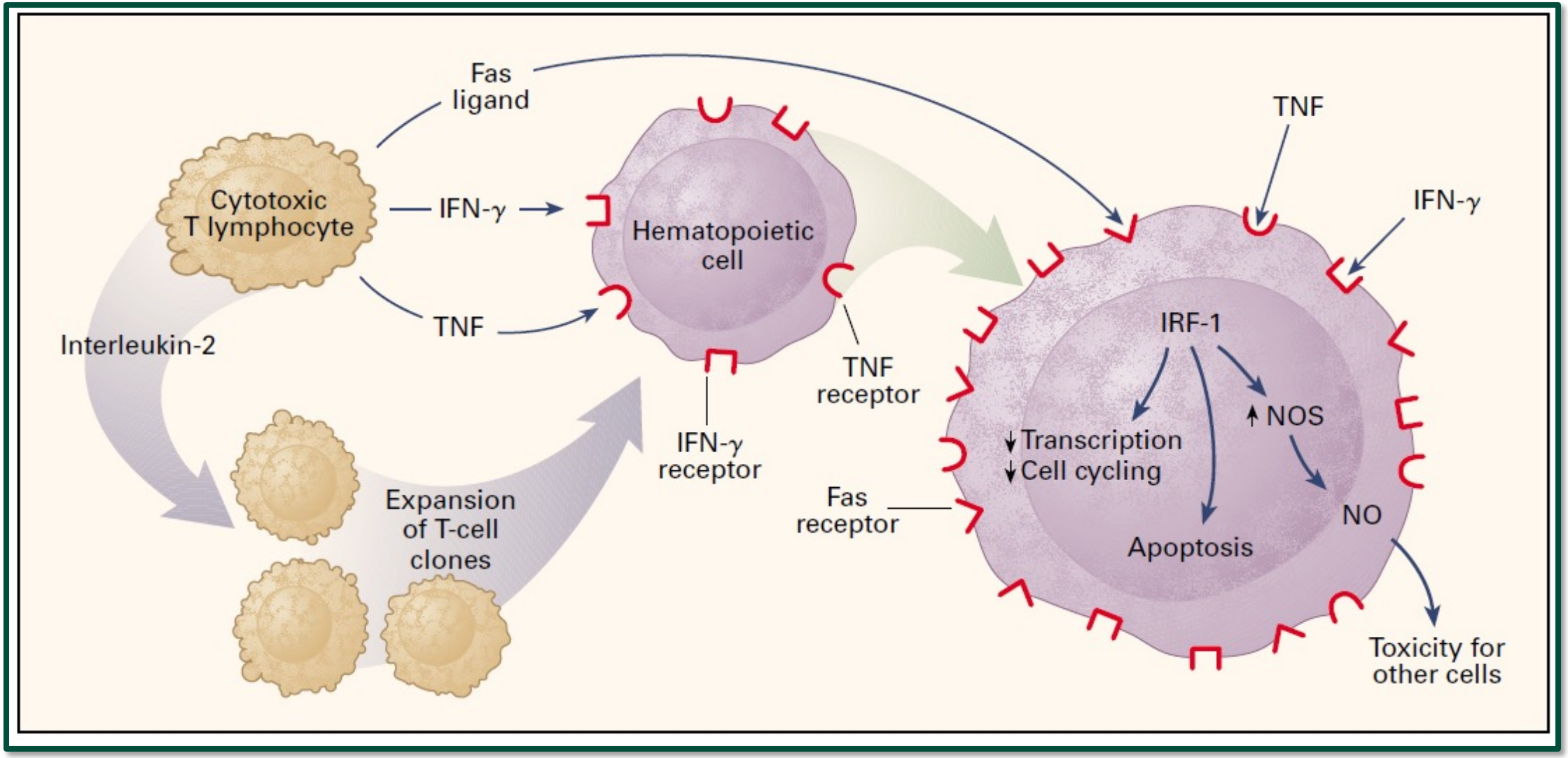
New perspectives

- The scenario is even more complex than thought!
- Predominant role of CTLs in marrow destruction, and type I interferons (IFNs) polarizing the immune system toward Th1 responses.
- Effector memory CD8+CD28–CD57+ T lymphocytes are frequently expanded in BMF and could mediate BM destruction.
- CD4+CD25highFoxP3+ T regulatory cells (Tregs) are decreased, while Th17 might be expanded in severe AA.



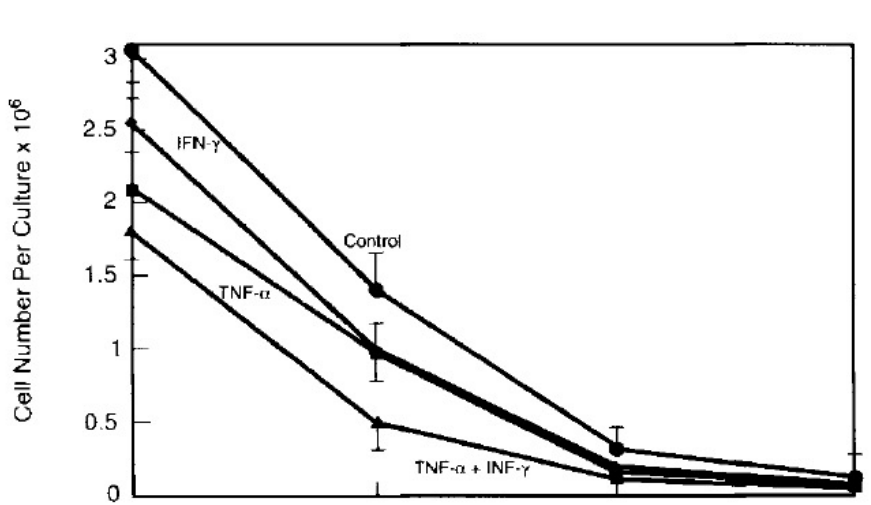
Giudice V, et al. Int J Mol Sci. 2021 Jan 12;22(2):705.

Back in ‘90s… the predominant role of IFN-γ



Selleri C, et al. J Cell Physiol. 1995 Dec;165(3):538-46.

IFN-γ: the principal BM blocker



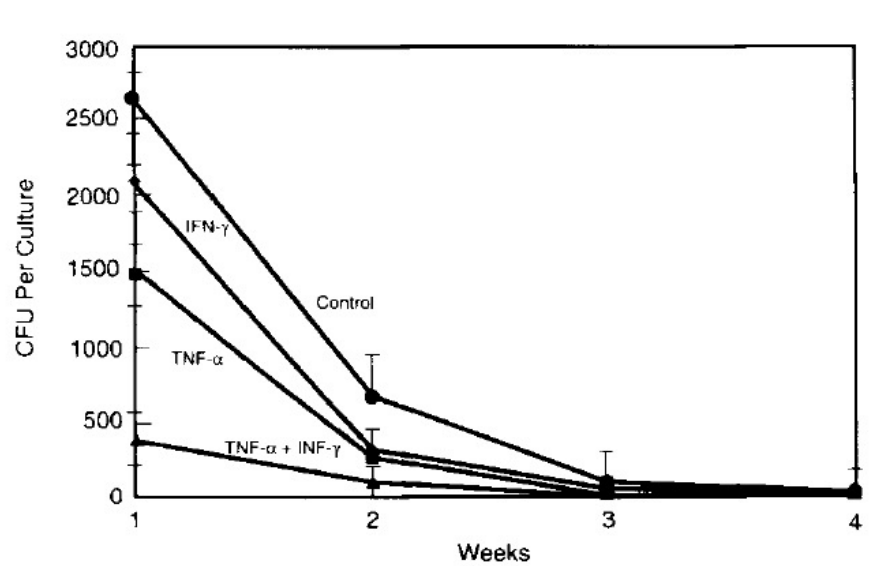


TABLE 2. Effects of TNF-α and IFN-γ on the maintenance of LTCIC in LTBMC[1]

	Control	IFN-γ	TNF-α	IFN-γ + TNF-α
No. of LTCIC per 10^5 cells	18 ± 4	8 ± 4	12 ± 3	3.5 ± 5
No. of LTCIC per culture	136 ± 12	18 ± 8	51 ± 13	10 ± 10

[1]Numbers represent results from six independent experiments. Each culture was performed in duplicate. IFN-γ and TNF-α were added together with fresh media at concentrations of 1,000 U/ml and 10 ng/ml per week, respectively. Paired *t* test: control vs. IFN-γ $P < .001$; control vs. TNF-α $P < .001$; TNF-α vs. IFN-γ + TNF-α $P < .01$; IFN-γ + TNF-α vs. IFN-γ $P < .01$.

TABLE 3. Effects of TNF-α and IFN-γ on the capability of $CD34^+$ cell population to generate LTCIC in LTBMC[1]

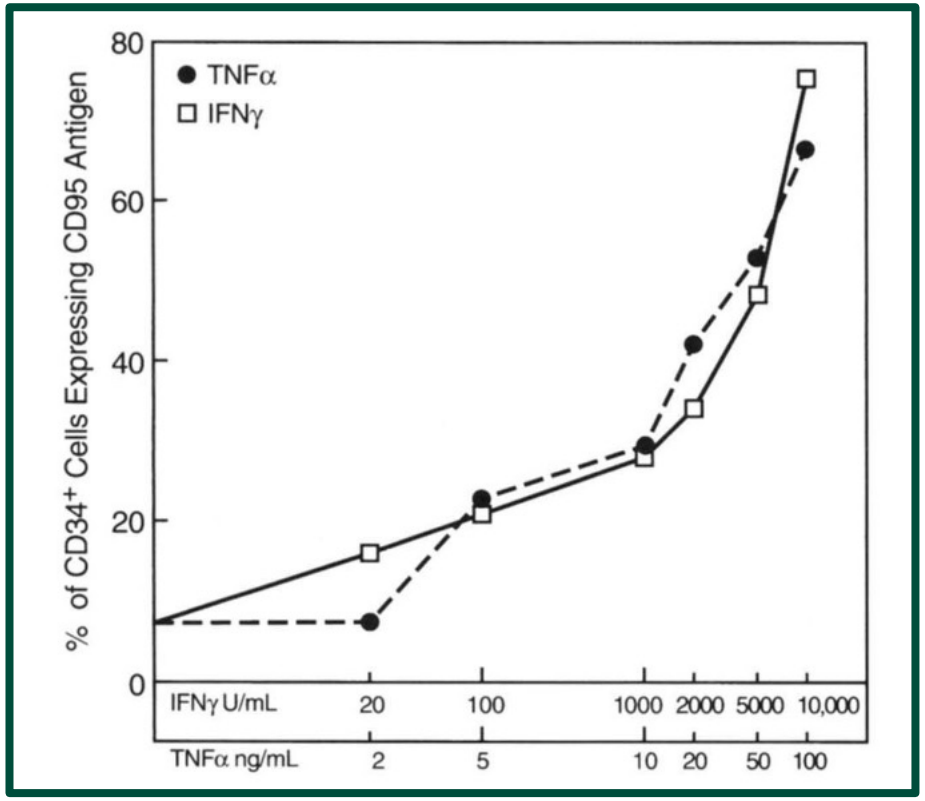
	Control	IFN-γ	TNF-α
No. of LTCIC per			
1×10^4	49 ± 4	6 ± 2	21 ± 3
5×10^3	28 ± 3	3 ± 1	15 ± 3
1×10^3	6 ± 2	0	2 ± 1
5×10^2	3 ± 2	0	0
1×10^2 $CD34^+$ cells	1 ± 1	0	0

[1]Values represent mean numbers ± SD of LTCIC. A total of two experiments were performed. Each experiment was performed in triplicate. Decreasing numbers (10^4, 10^3, 10^2 cells per well) of $CD34^+$ cells (89% and 95% purity) were plated on preformed irradiated allogeneic stroma. IFN-γ and TNF-α were added together with fresh media at concentration of 1,000 U/ml and 10 ng/ml per week, respectively.

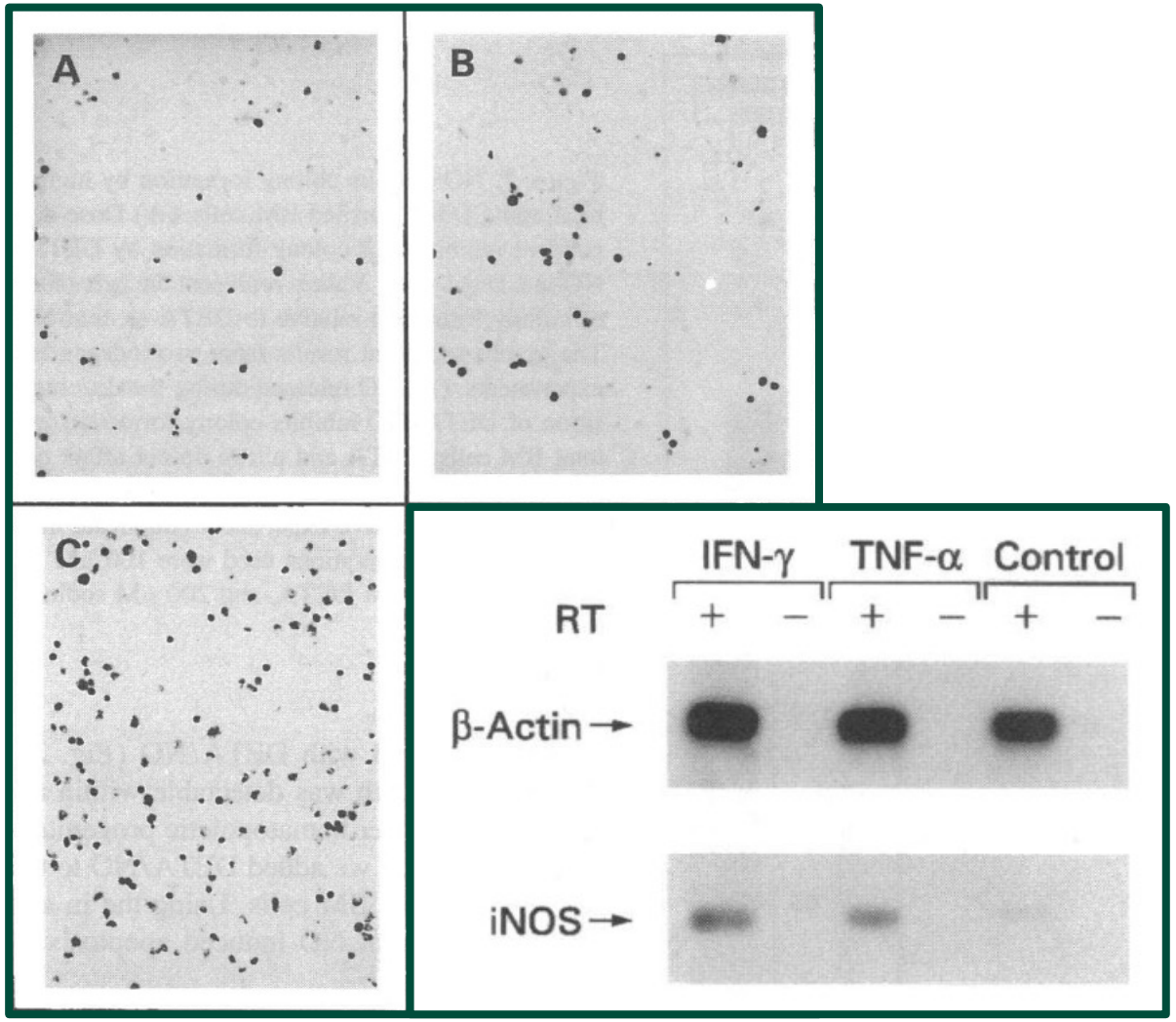
Selleri C, et al. J Cell Physiol. 1995 Dec;165(3):538-46.

IFN-γ: the «kill-me» inducer on HSCs

Fas expression



iNOS expression



Maciejewski J, Selleri C, Anderson S, Young NS. Blood. 1995 Jun 1;85(11):3183-90.

Maciejewski JP, Selleri C, et al. J Clin Invest. 1995 Aug;96(2):1085-92.

Back in ‘90s… the predominant role of IFN-γ

Table 1. Deregulated cytokines in acquired aplastic anemia (AA).

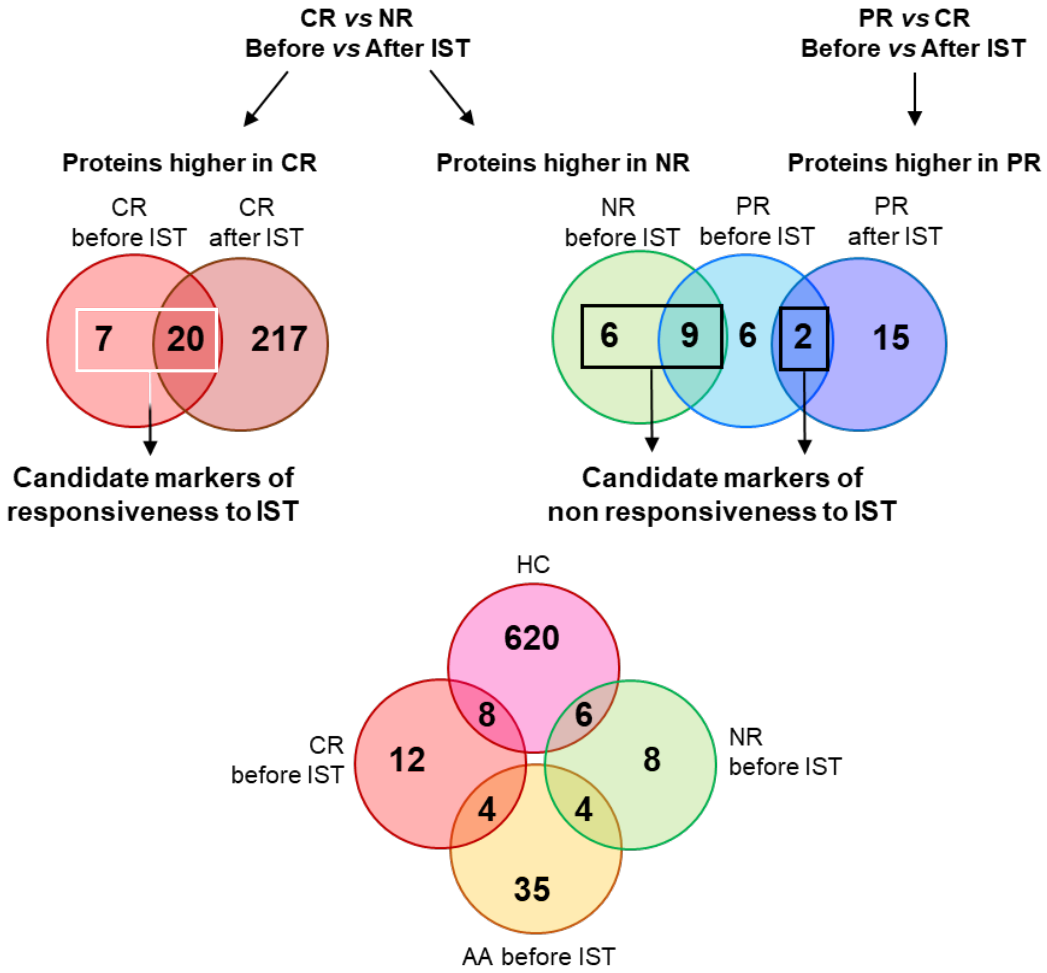
	ILs	Chemokines	IFNs/TNFs	Growth Factors	Others
Increased	IL-2 IL-8 IL-12 IL-17A IL-18 IL-21 IL-23	CXCL10 CCL20	IFN-γ TNFα	G-CSF TPO EPO	GDF-15 sST2
Decreased	IL-33 IL-35	CCL5 CCL11 CCL17 CXCL5 CXCL11		EGF VEGF	CD40L SELL DKK1 c-Mpl Hepcidin
No changes	IL-1Ra IL-6	CCL2 CCL3 CCL4 CXCL9 CXCL11		HGF (or slightly reduced)	S100A8 S100A9 S100A8/A9

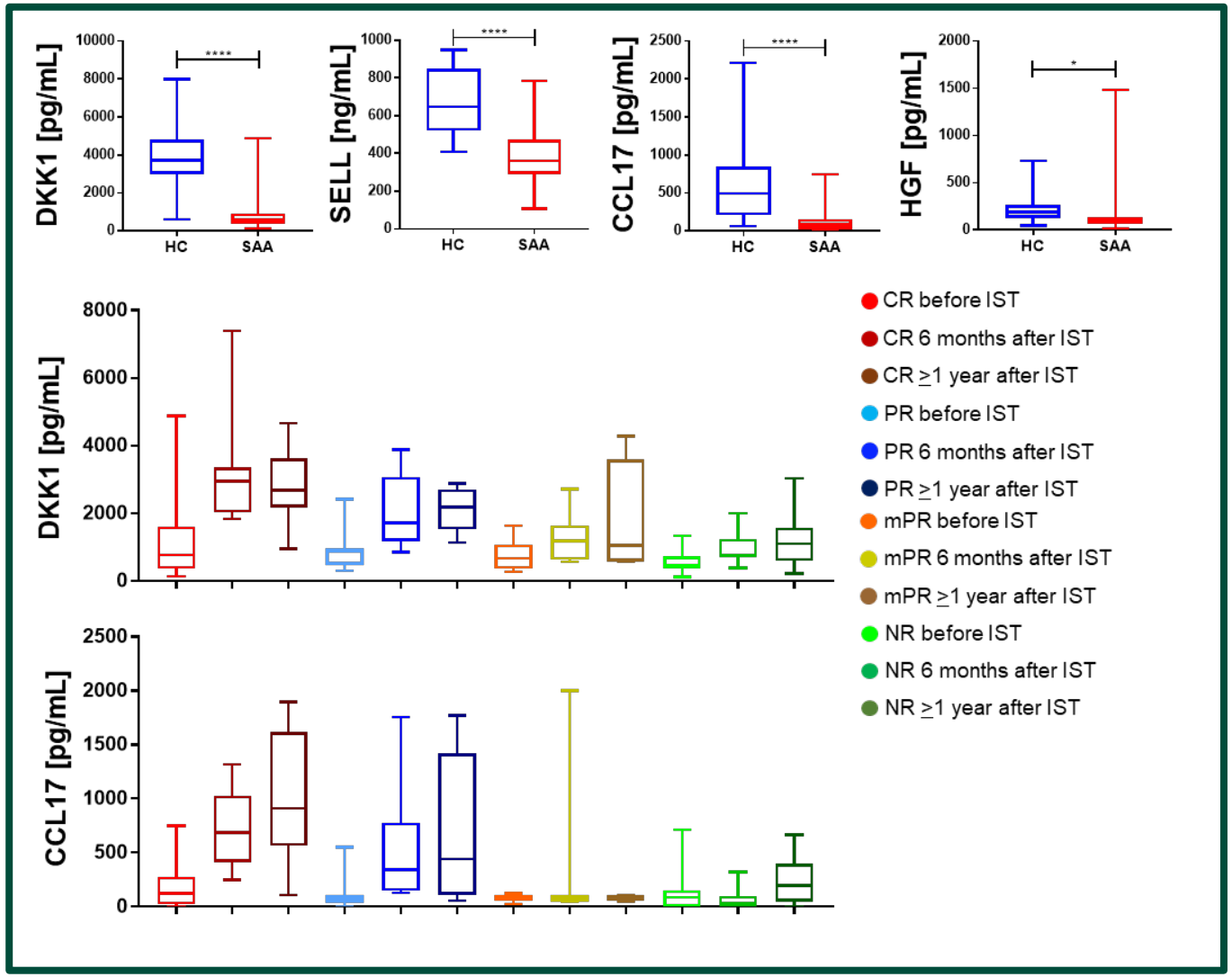
Abbreviations. ILs, interleukins; IFNs, interferons; TNFs, tumor necrosis factors; CCL, CC chemokine ligands; CXCL, C-X-C motif chemokine; G-CSF, granulocyte colony-stimulating factor; TPO, thrombopoietin; EPO, erythropoietin; EGC, epidermal growth factor; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; GDF, growth differentiation factor; SELL, L-selectin; DKK1, Dickkopf-related protein 1; c-Mpl, thrombopoietin receptor.

Giudice V, et al. Int J Mol Sci. 2021 Jan 12;22(2):705.

New perspectives and biological features

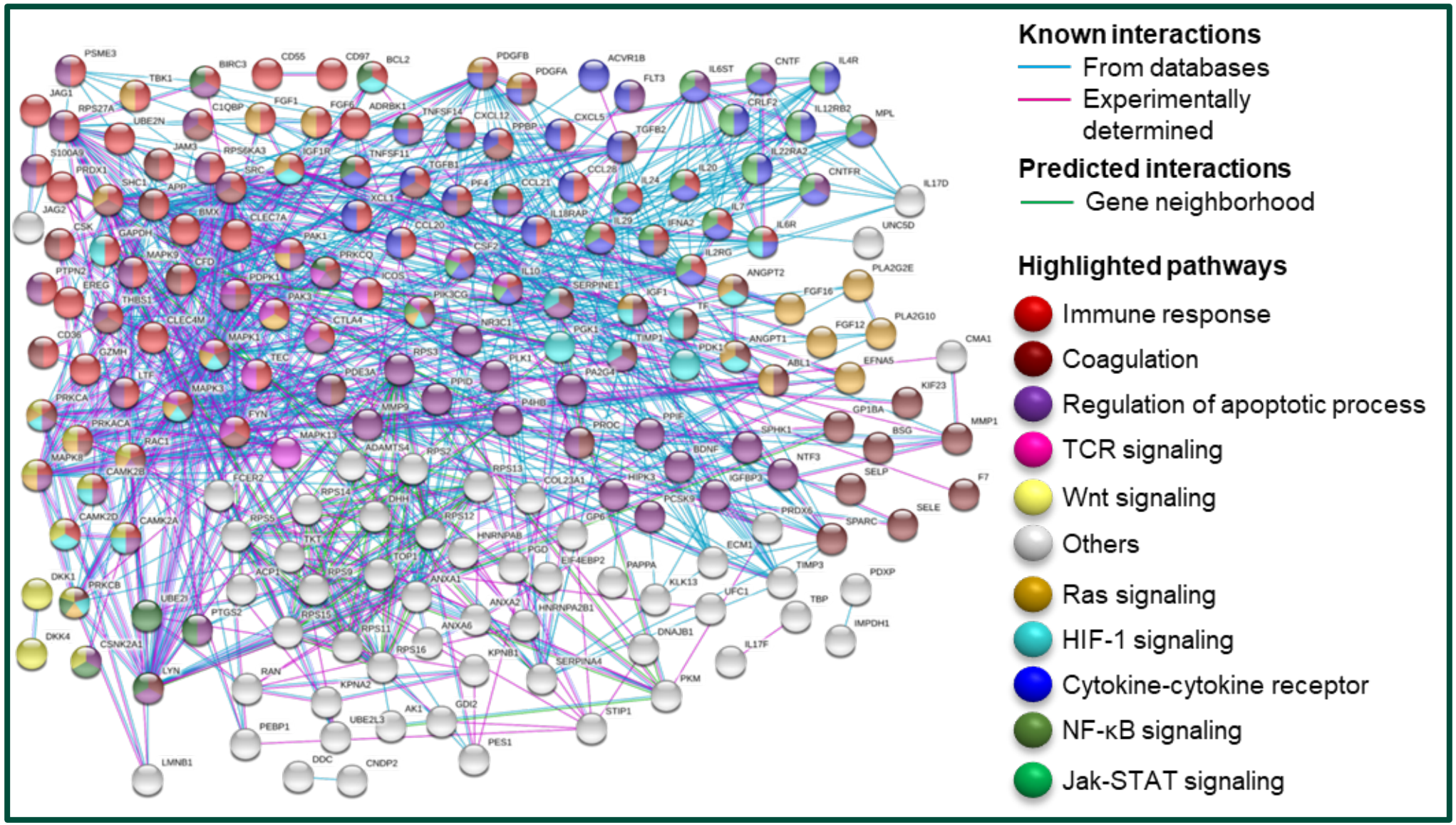
- IFN-γ and TNF-α are historically implicated in AA pathogenesis; however, several other proteins might be involved.





Giudice V, et al. Exp Hematol. 2018 Dec;68:38-50.

Digging in the mine of BMF data



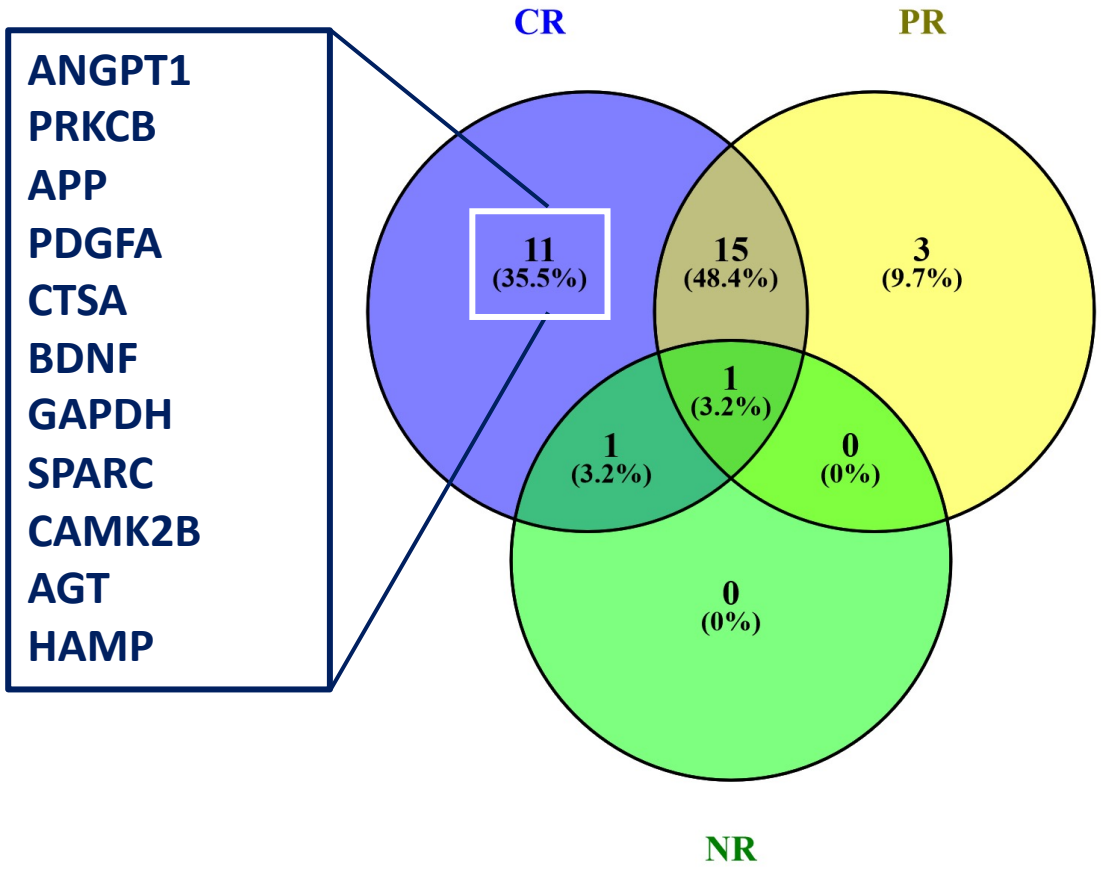
Giudice V, et al. Exp Hematol. 2018 Dec;68:38-50.

J. Pollock, 1952, Convergence

Treatment-modified proteins in AA

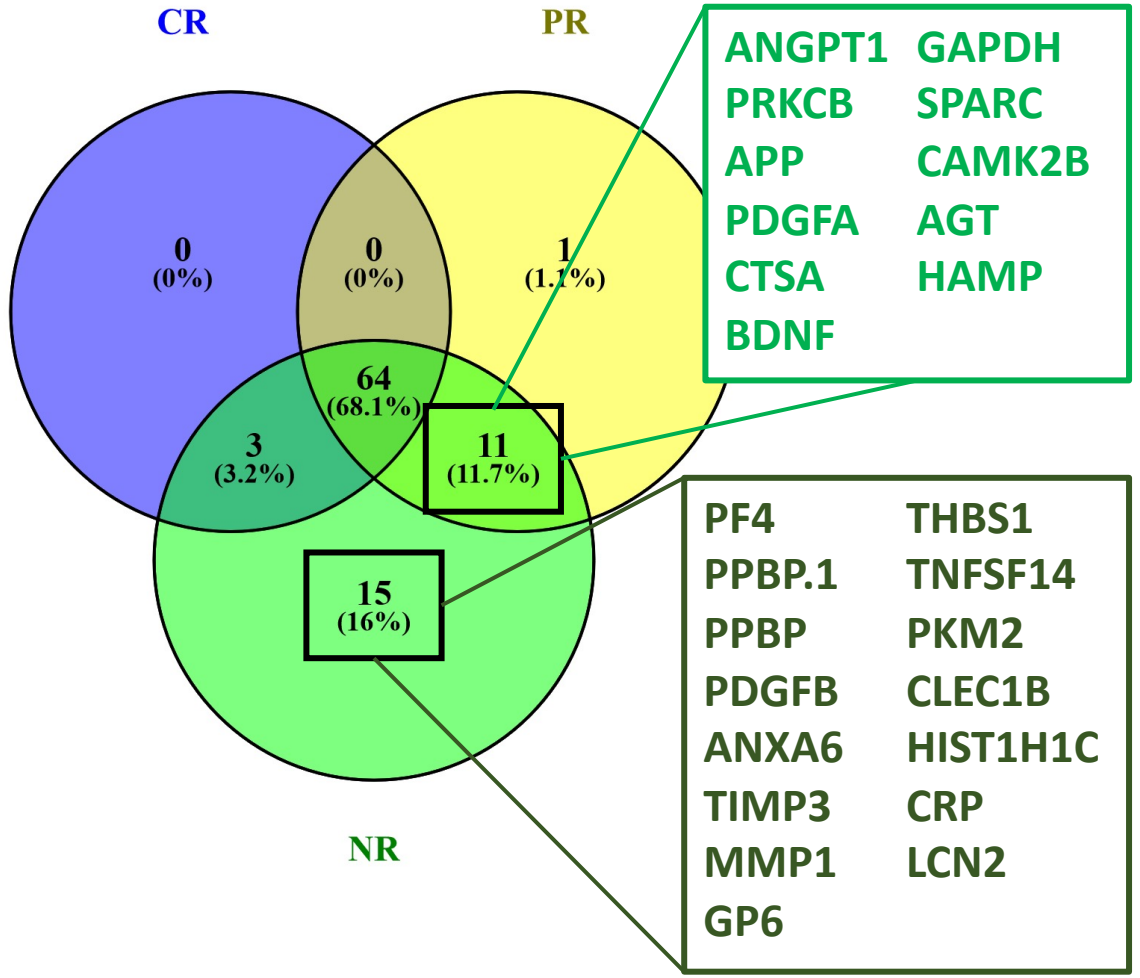
Before – after therapy

Treatment-modified proteins



Before – after therapy

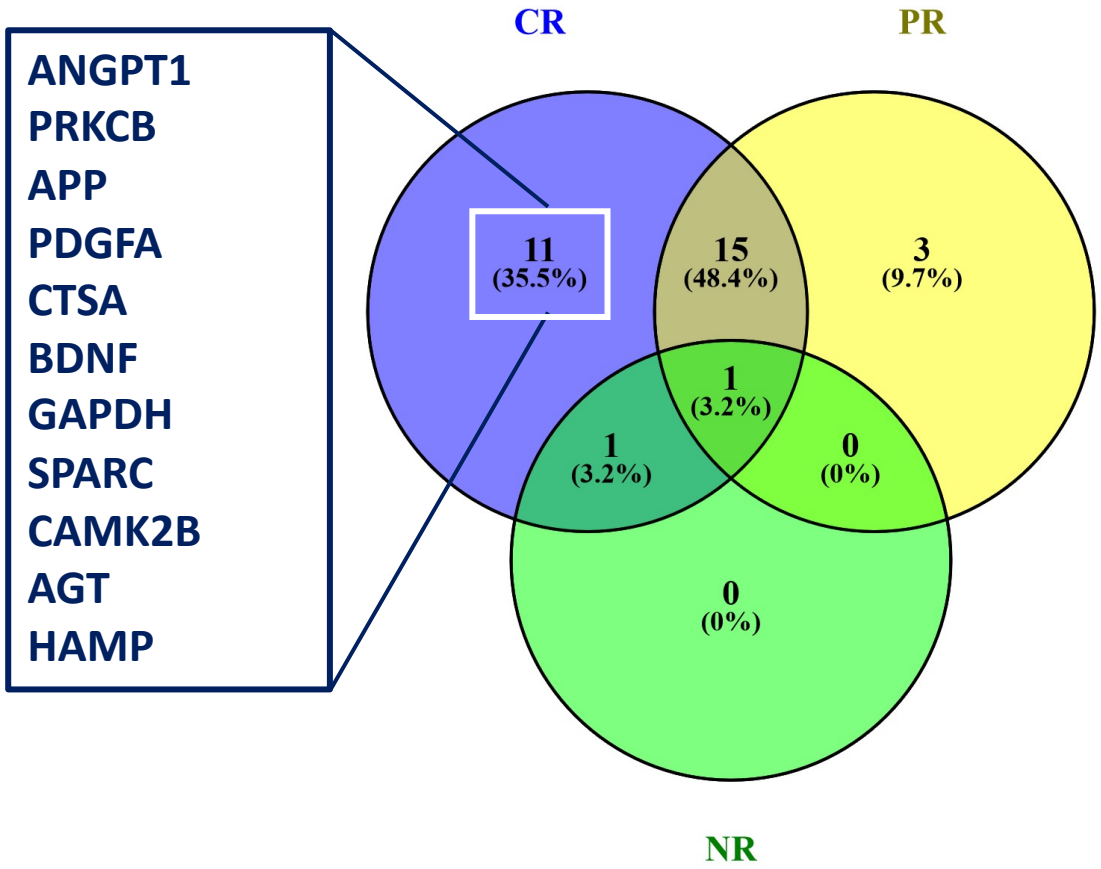
Unmodified proteins



Treatment-modified proteins in CR and PR

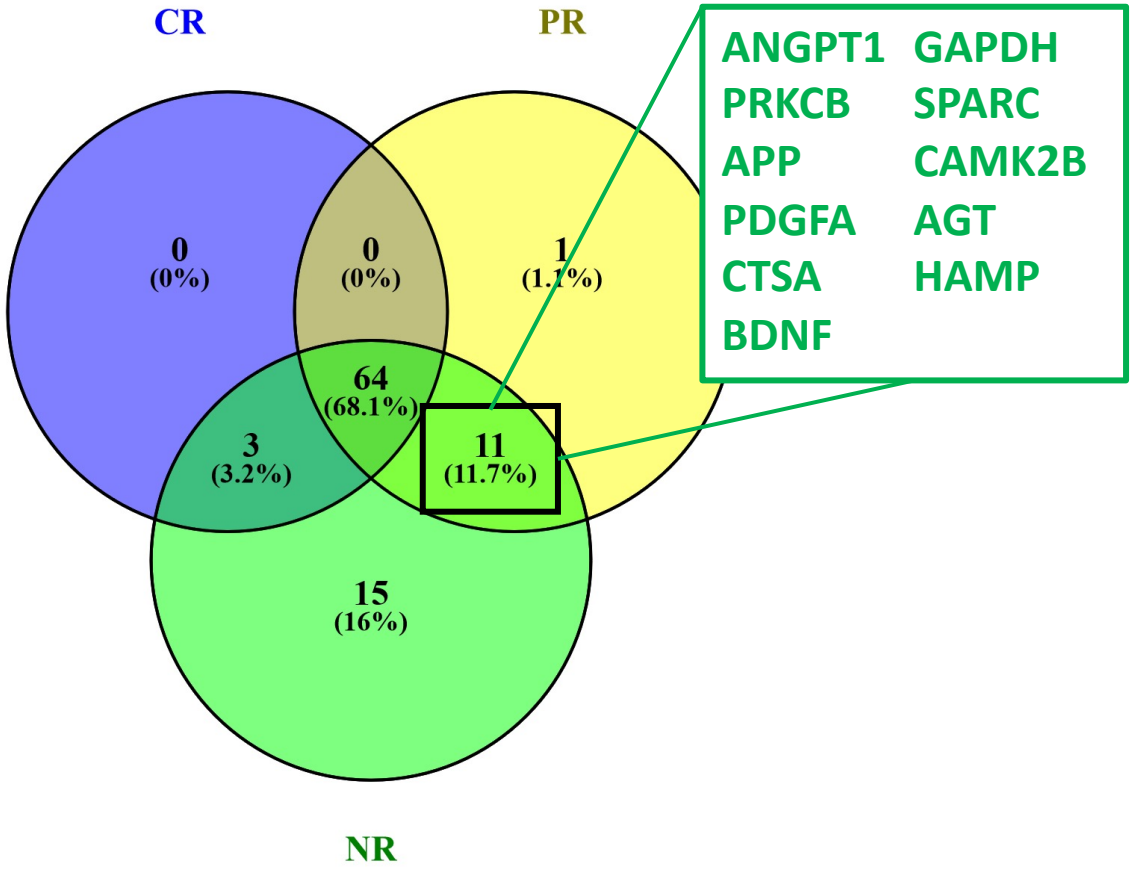
Before – after therapy

Treatment-modified proteins



Before – after therapy

Unmodified proteins

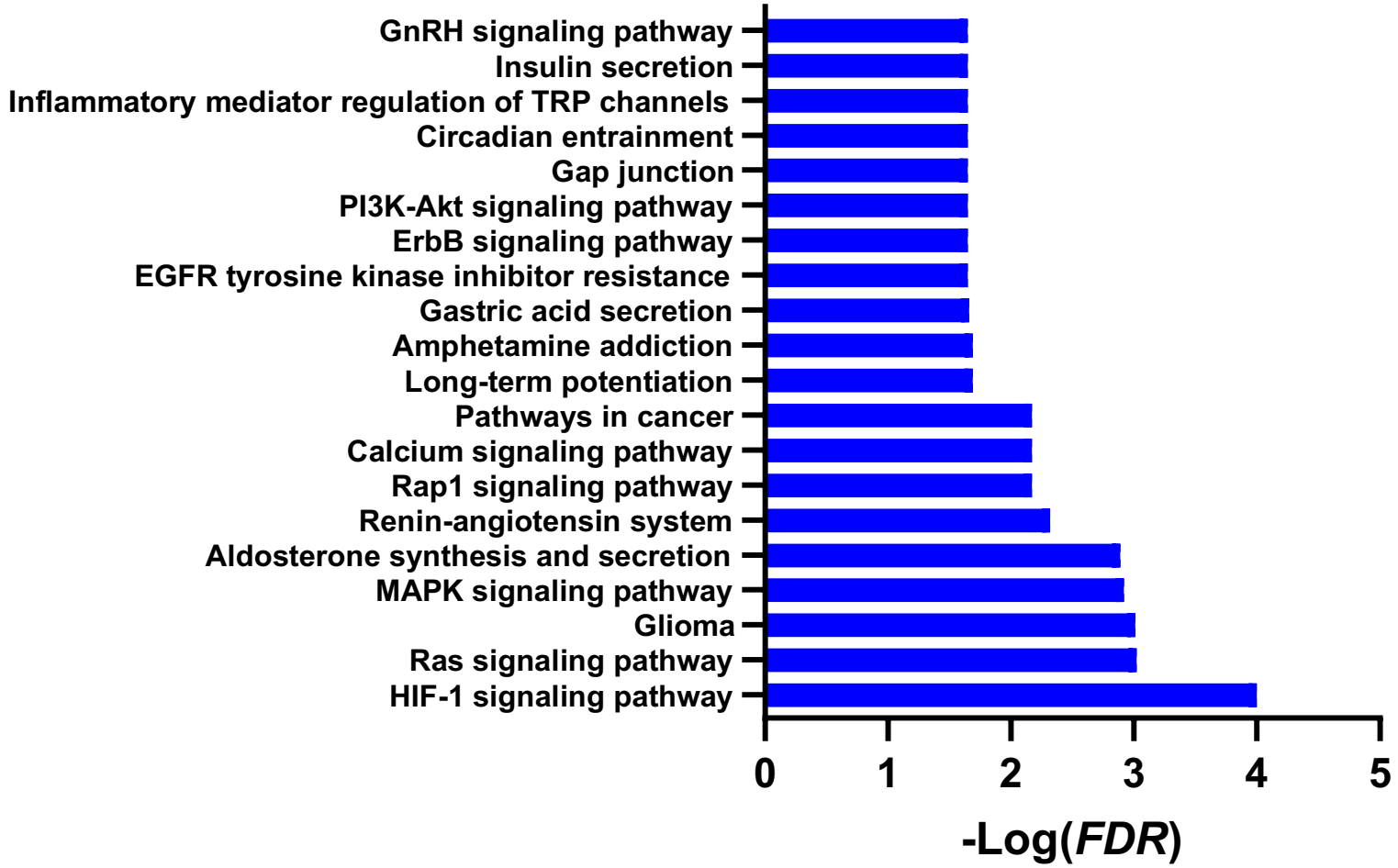


Pathways in CR and PR

Before – after therapy

Treatment-modified proteins

ANGPT1
PRKCB
APP
PDGFA
CTSA
BDNF
GAPDH
SPARC
CAMK2B
AGT
HAMP

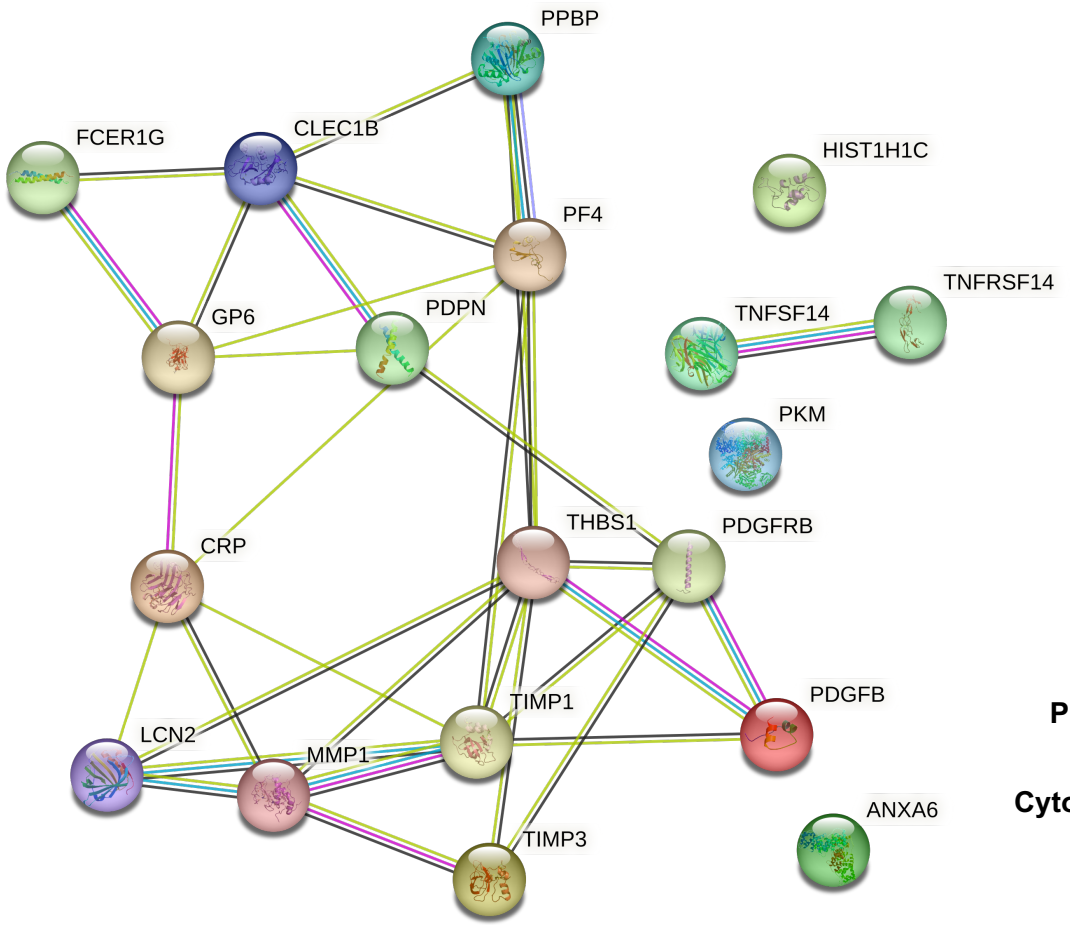


Before – after therapy

Unmodified proteins

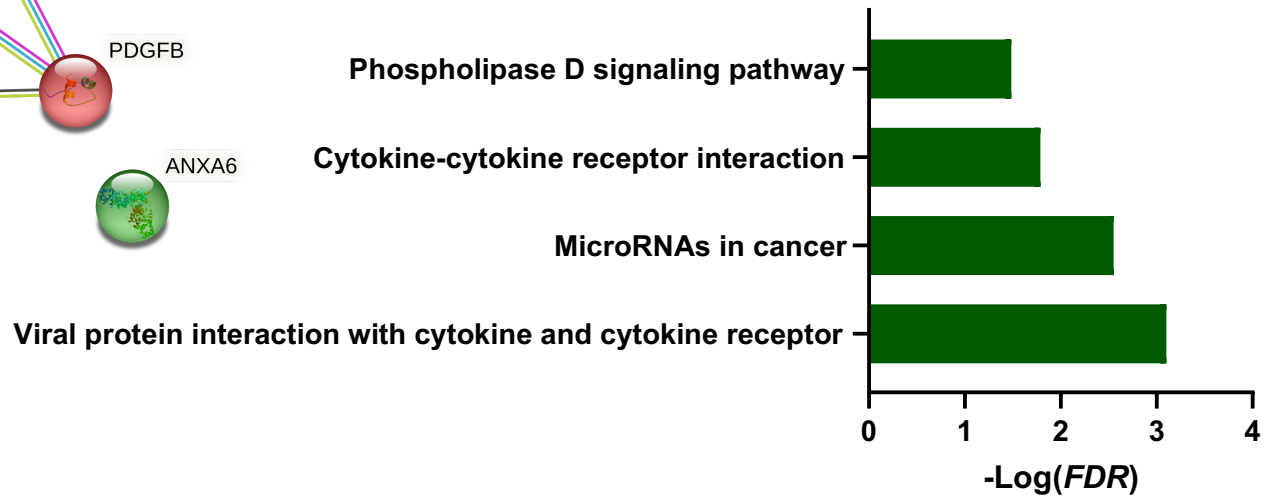
ANGPT1 GAPDH
PRKCB SPARC
APP CAMK2B
PDGFA AGT
CTSA HAMP
BDNF

Proteins and pathways in NR



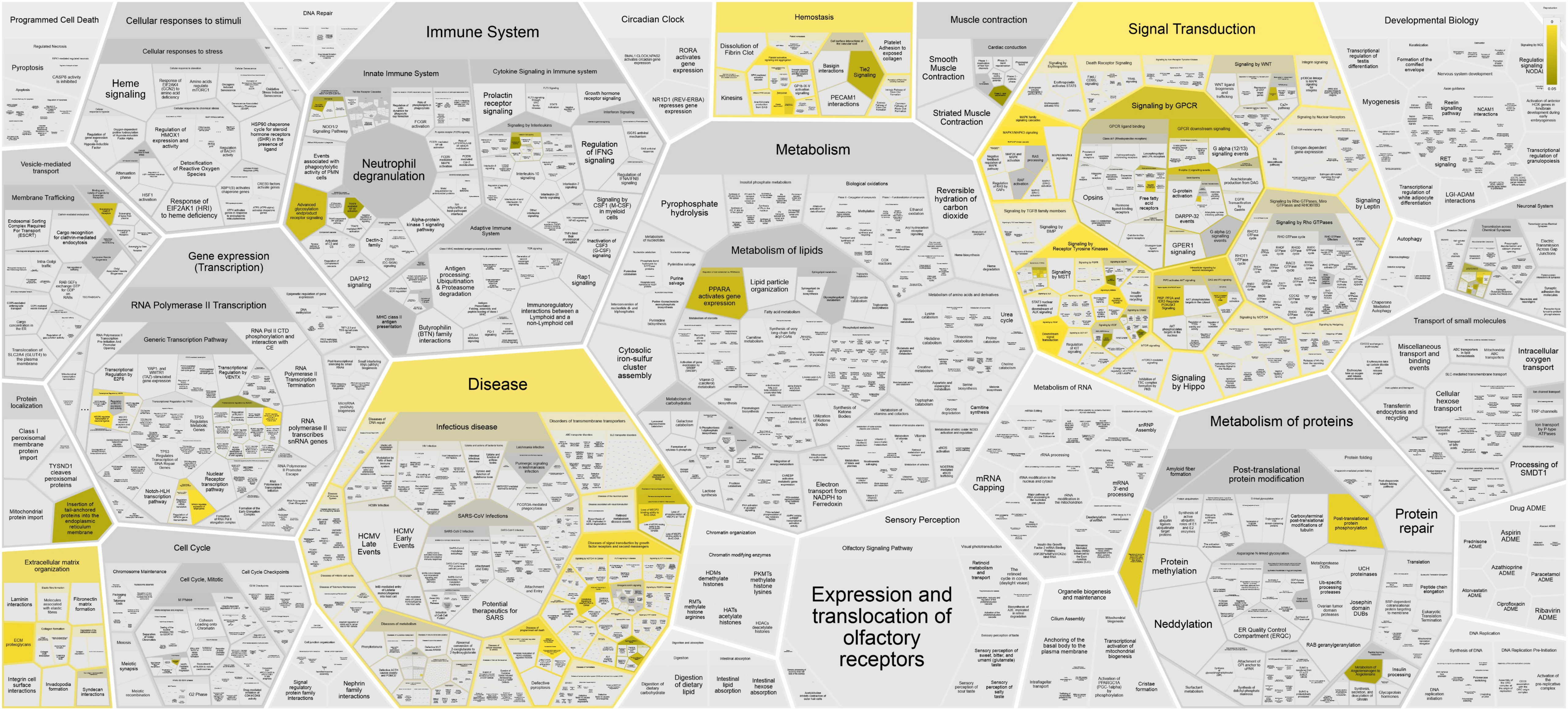
Before – after therapy

Unmodified proteins

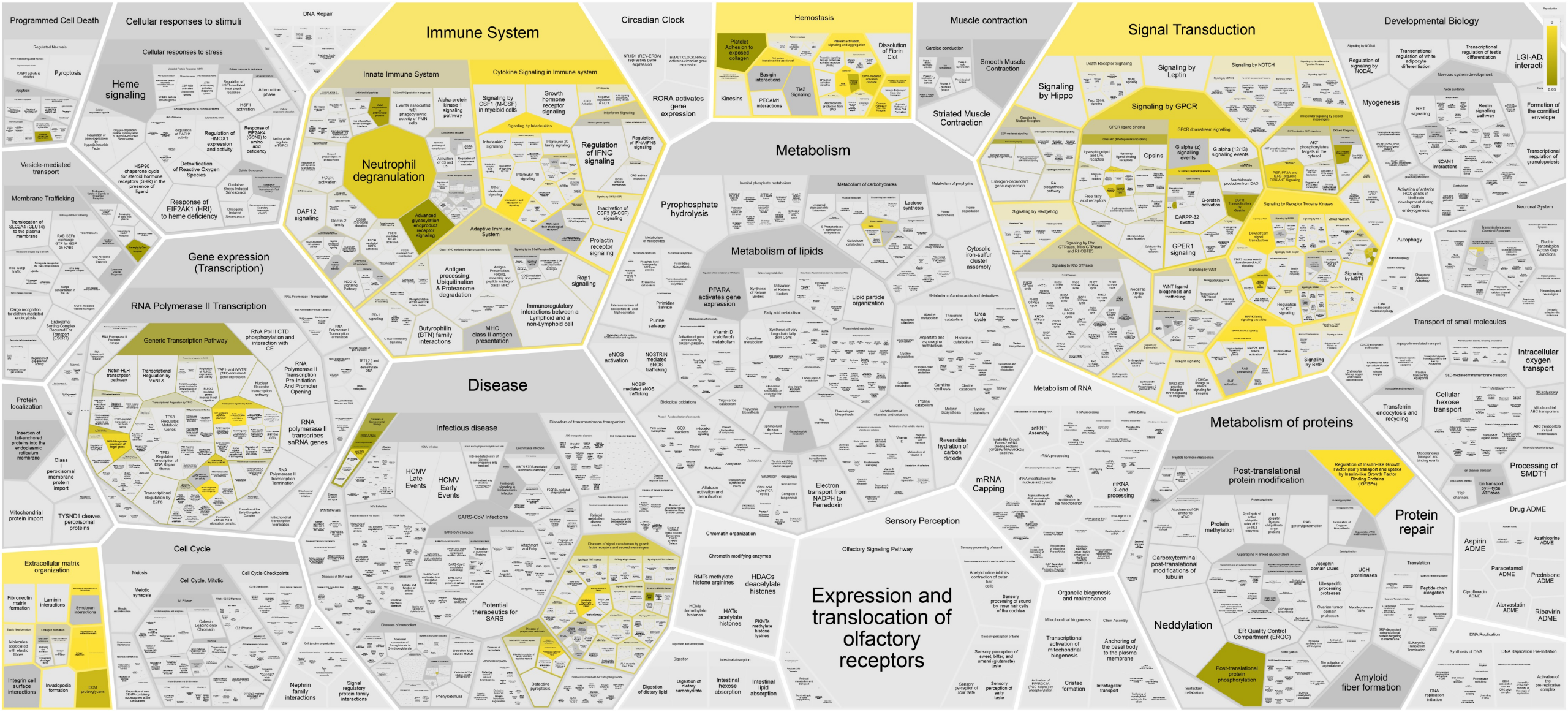


PF4	THBS1
PPBP.1	TNFSF14
PPBP	PKM2
PDGFB	CLEC1B
ANXA6	HIST1H1C
TIMP3	CRP
MMP1	LCN2
GP6	

Protein profiling in CR



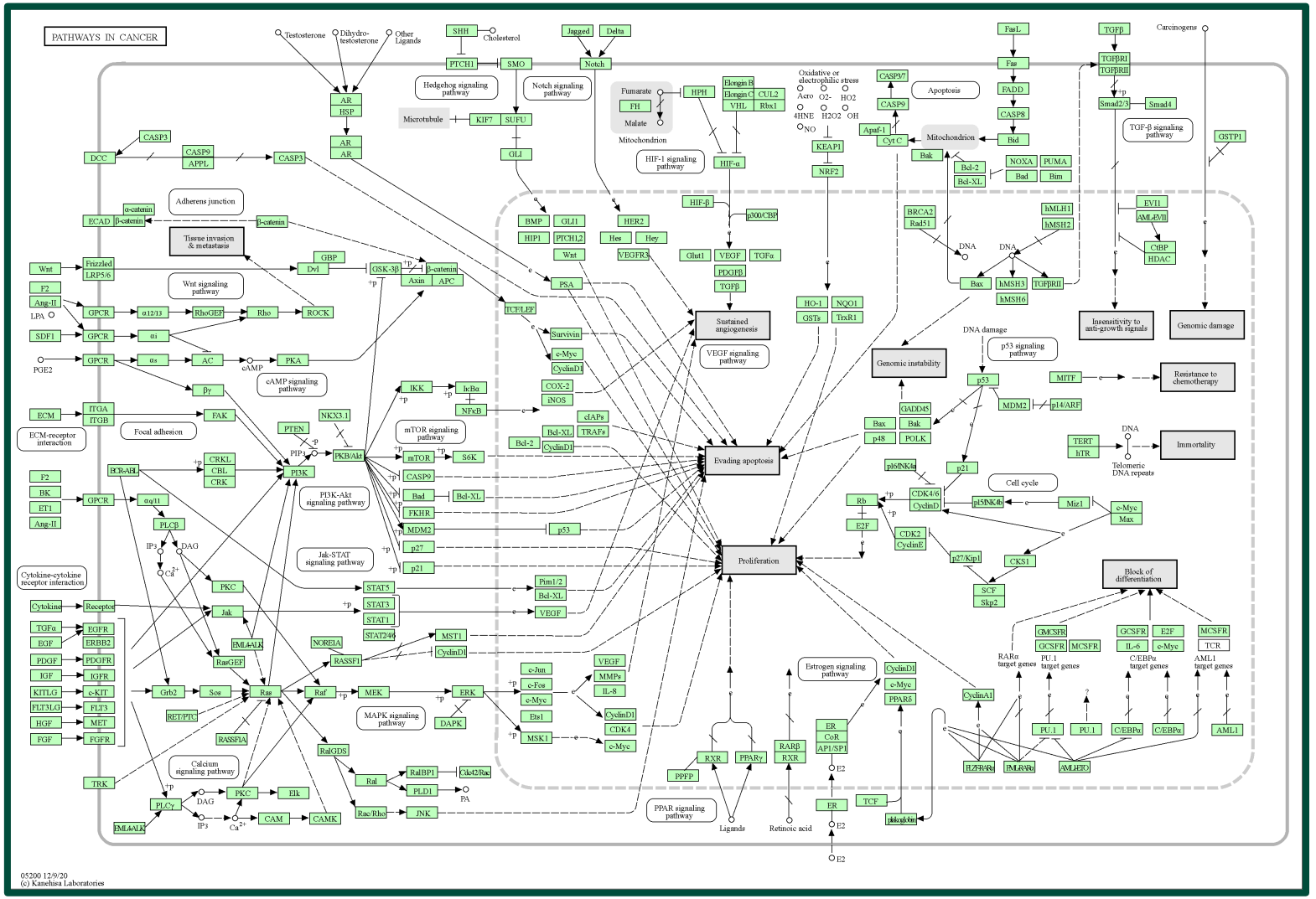
Protein profiling in NR / PR



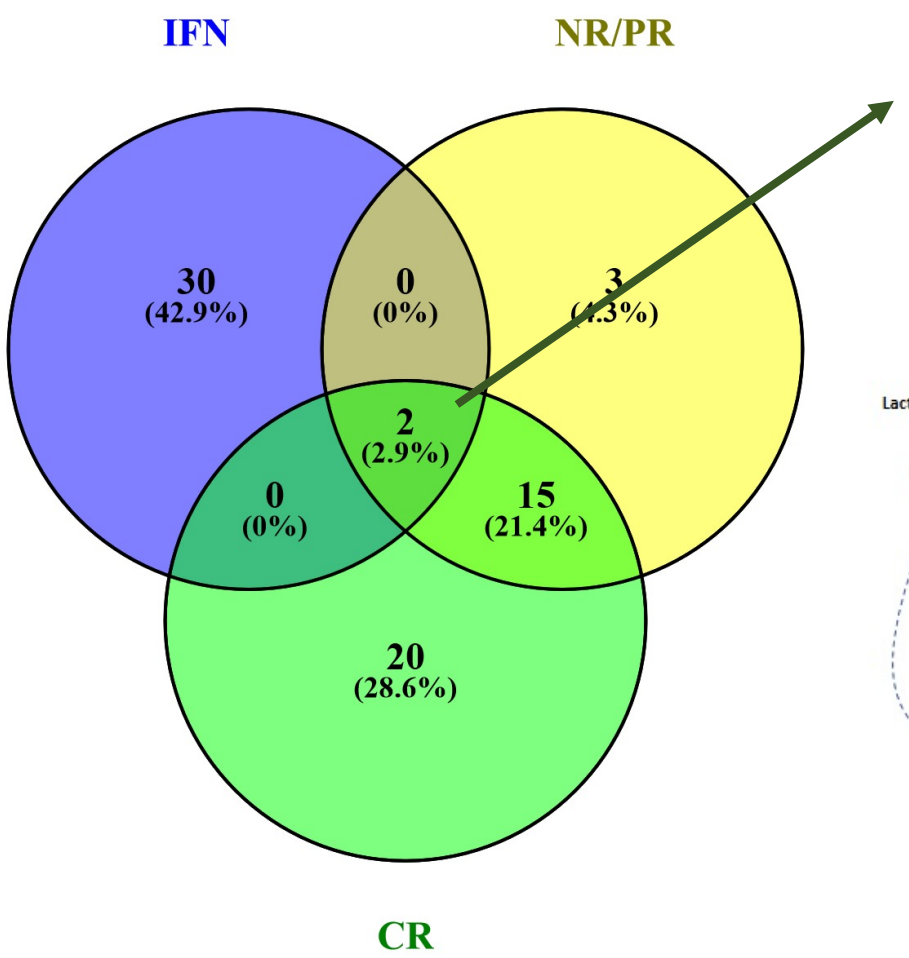
IFN-γ: from new to old-fashioned molecules

IFN-γ-related pathways

Proteasome
Cytokine-cytokine receptor interaction
HIF-1 signaling pathway
Necroptosis
TGF-beta signaling pathway
Antigen processing and presentation
JAK-STAT signaling pathway
Natural killer cell mediated cytotoxicity
IL-17 signaling pathway
Th1 and Th2 cell differentiation
Th17 cell differentiation
T cell receptor signaling pathway
Autoimmune disorders
Infectious diseases
Pathways in cancer
PD-L1 expression and PD-1 checkpoint
Allograft rejection
Graft-versus-host disease
Fluid shear stress and atherosclerosis

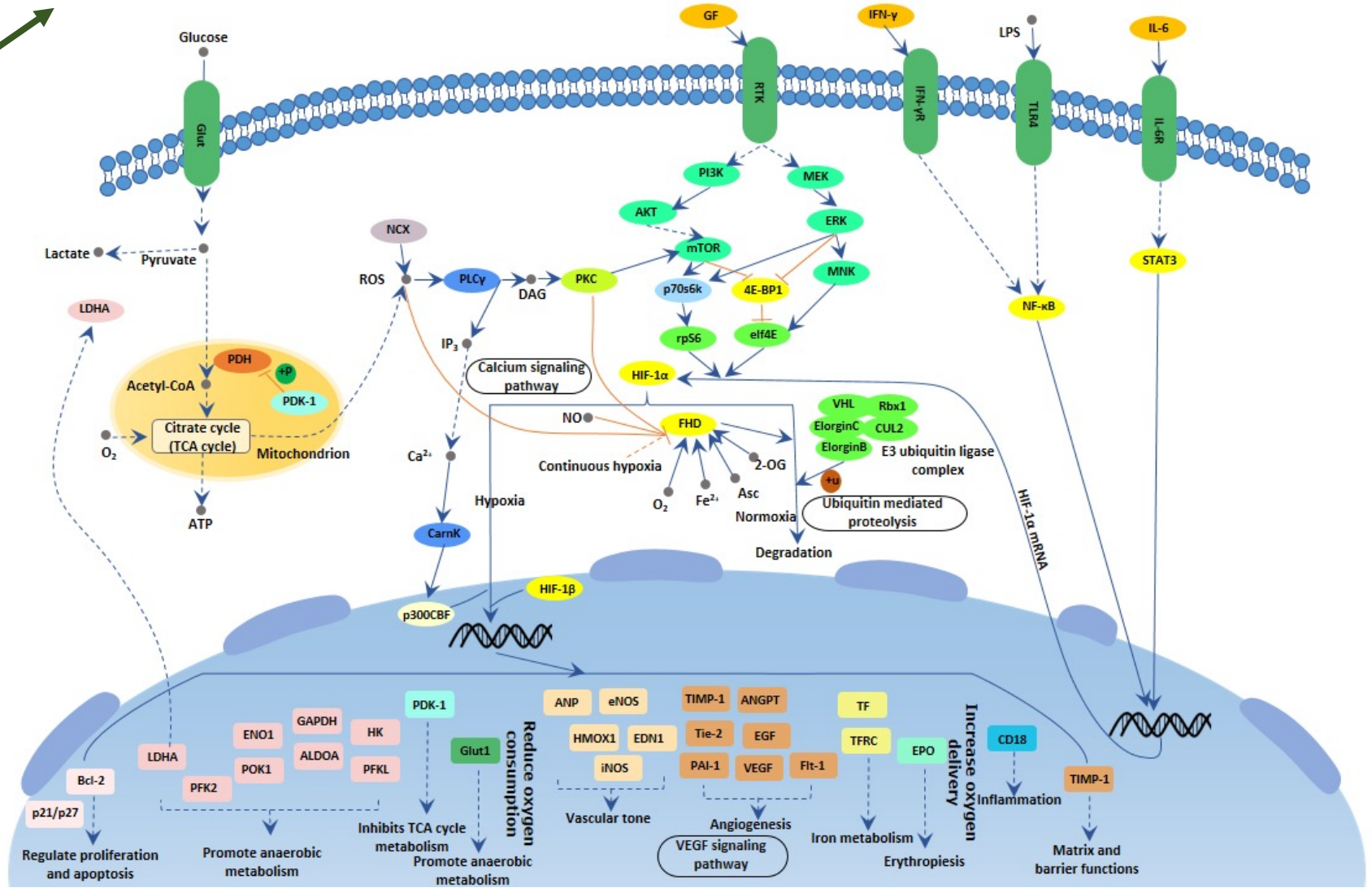


IFN-γ: from new to old-fashioned molecules

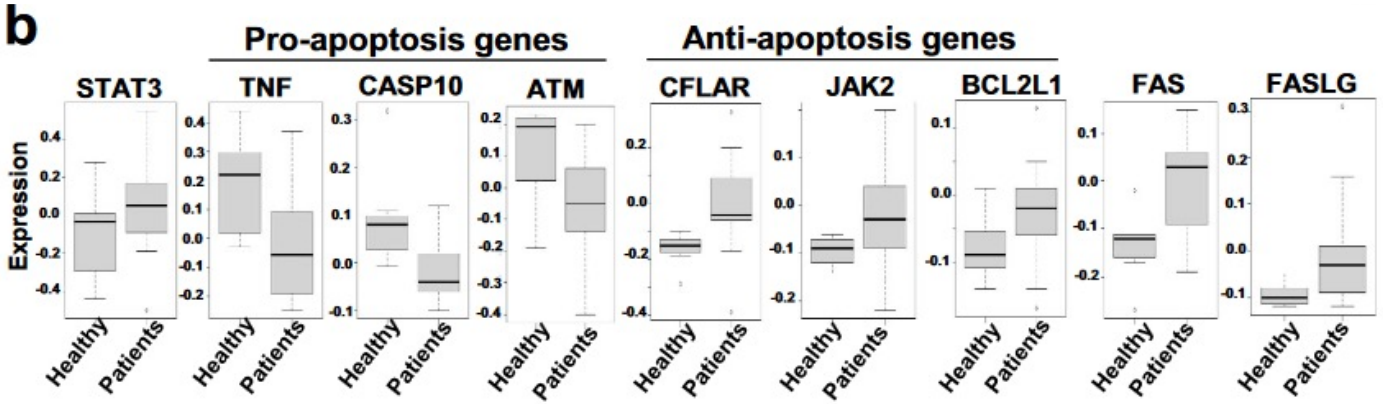


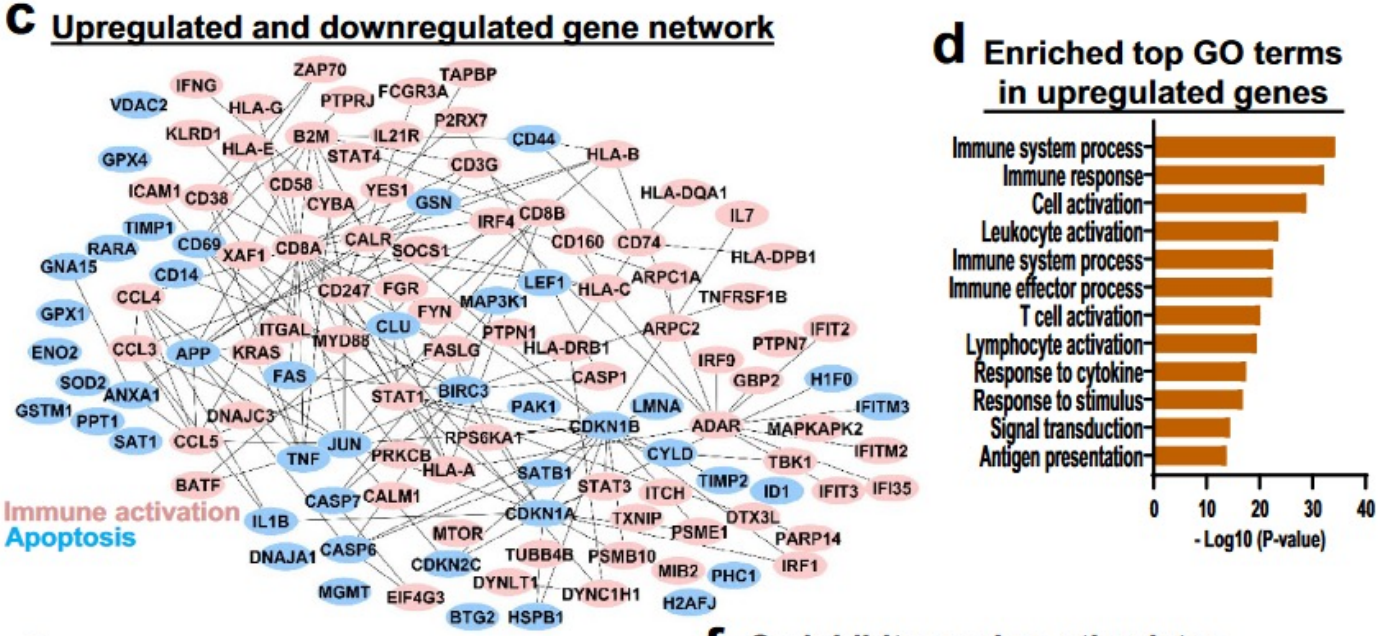
HIF-1 signaling pathway

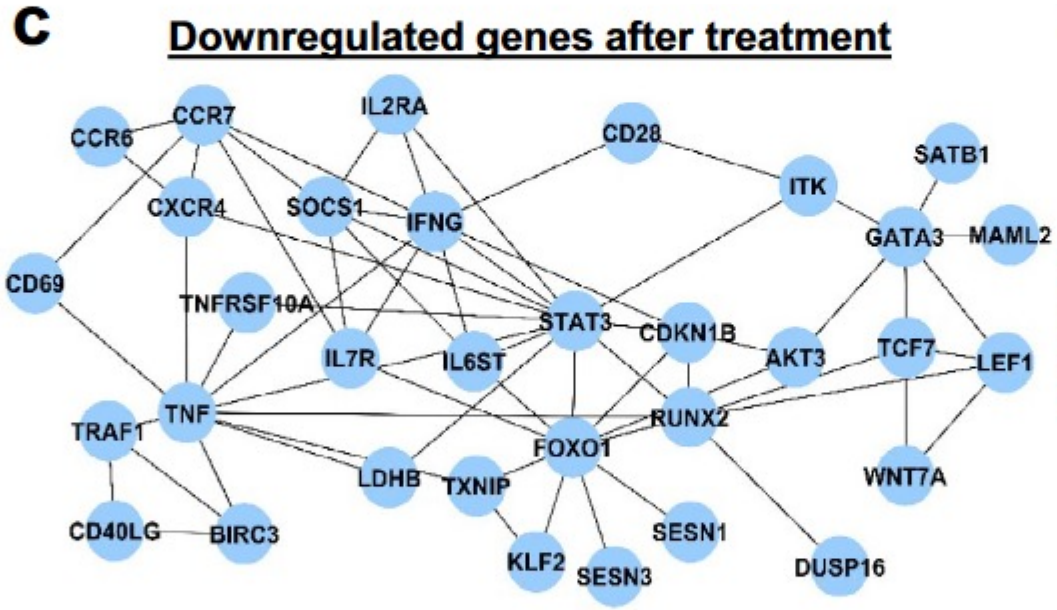
Pathways in cancer



IFN-γ: from a different point of view - LGL

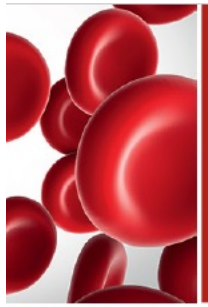






Gao S, et al. Nat Commun. 2022 Apr 11;13(1):1982.

Targeting IFN-γ pathways – JAK1/2



blood®

Regular Article

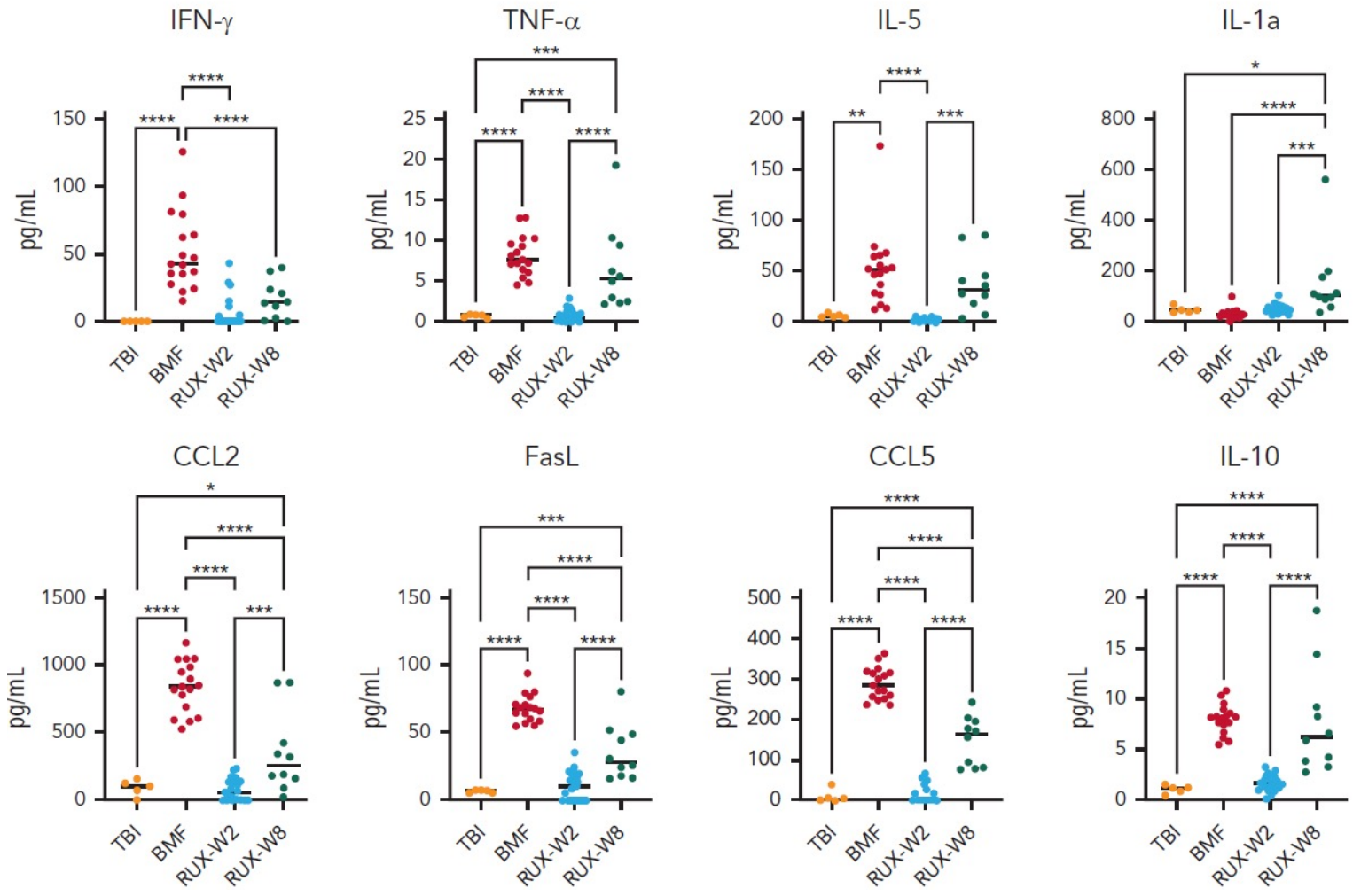
HEMATOPOIESIS AND STEM CELLS

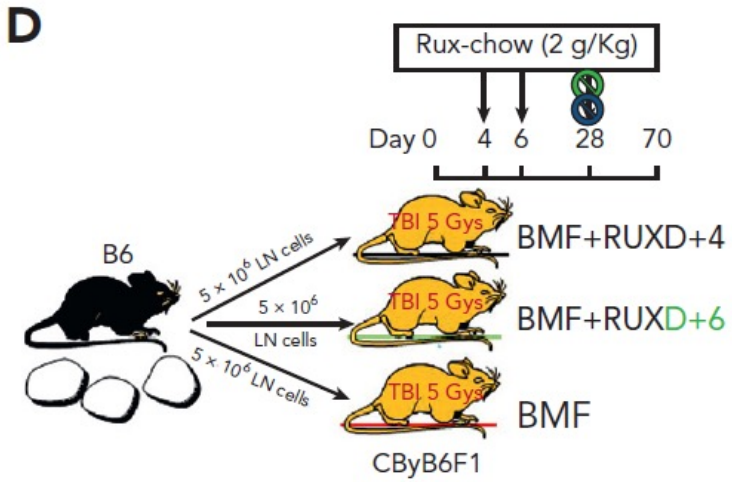
Efficacy of JAK1/2 inhibition in murine immune bone marrow failure

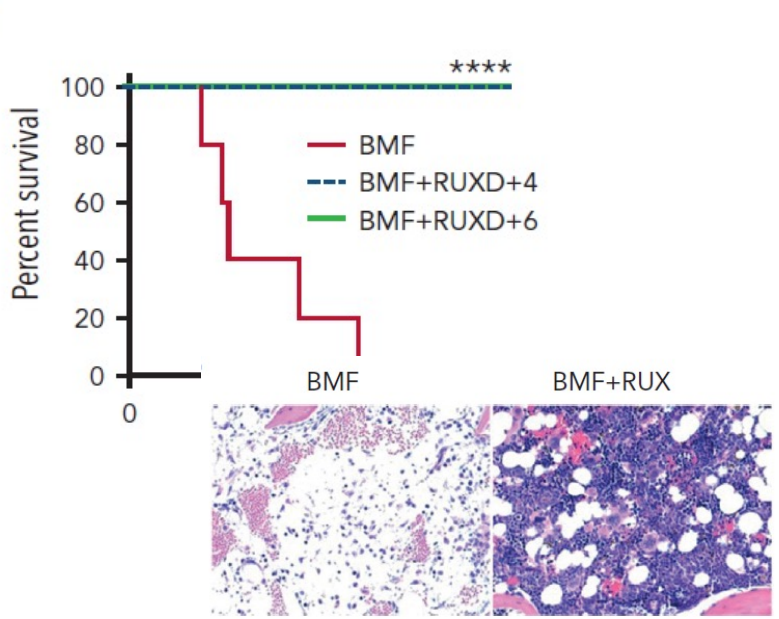
Emma M. Groarke, Xingmin Feng, Nidhi Aggarwal, Ash Lee Manley, Zhijie Wu, Shouguo Gao, Bhavisha A. Patel, Jichun Chen, and Neal S. Young

Groarke EM, et al. Blood. 2023 Jan 5;141(1):72-89.

Targeting IFN-γ pathways – JAK1/2 & cytokines

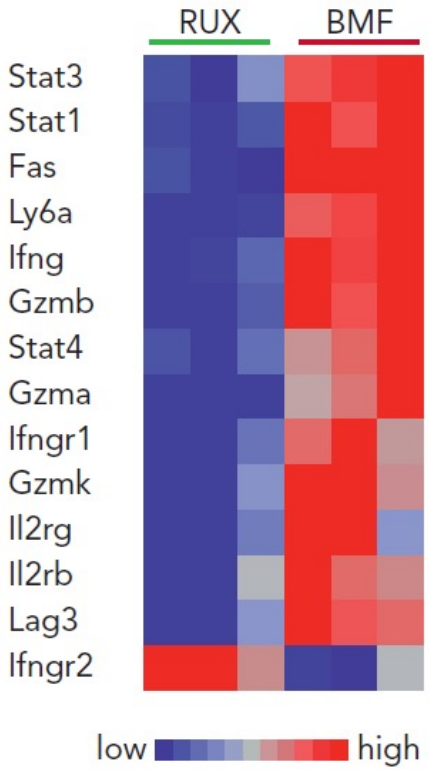


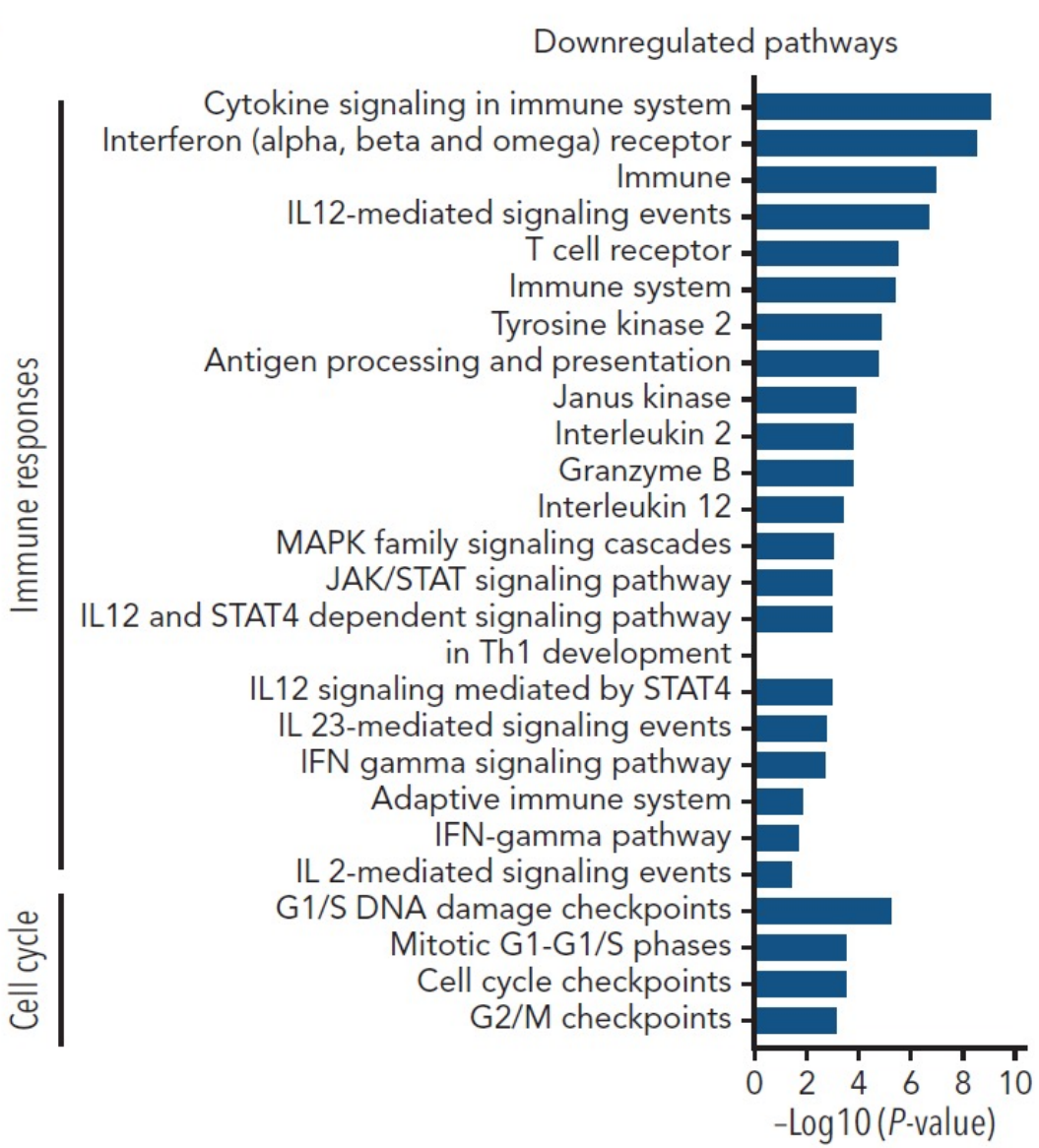




Groarke EM, et al. Blood. 2023 Jan 5;141(1):72-89.

Targeting IFN-γ pathways – JAK1/2 & pathways

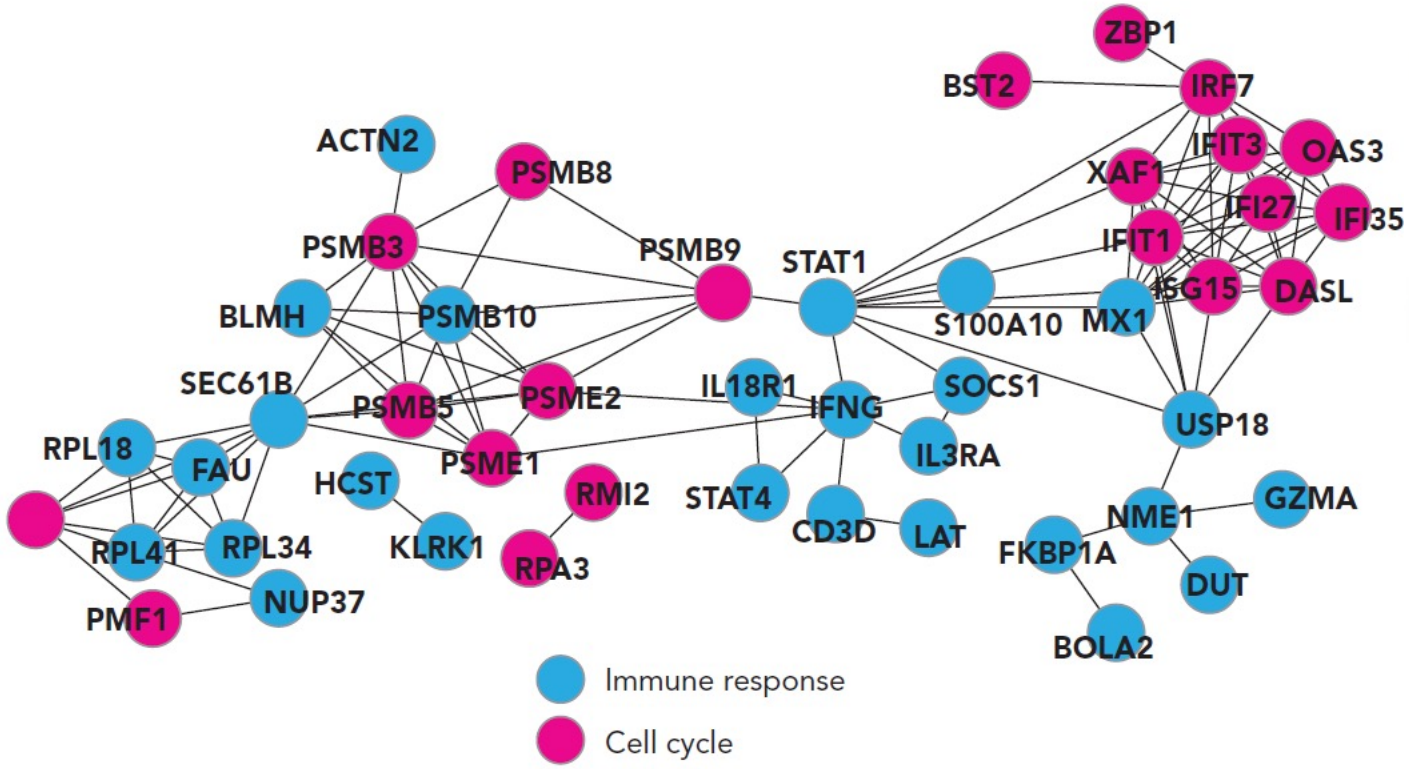


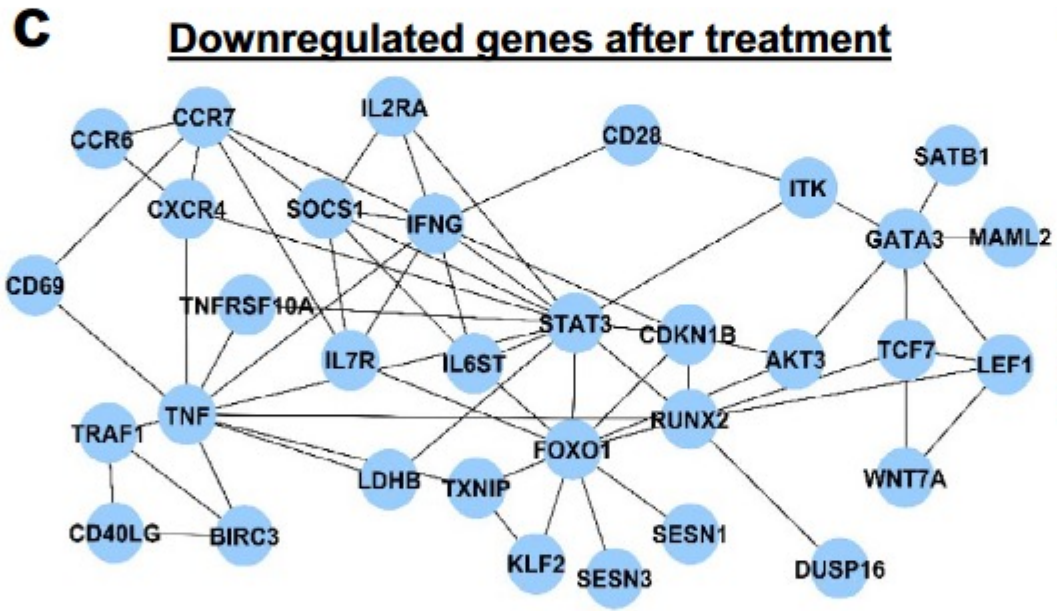


Groarke EM, et al. Blood. 2023 Jan 5;141(1):72-89.

Targeting IFN-γ pathways – JAK1/2 & genes

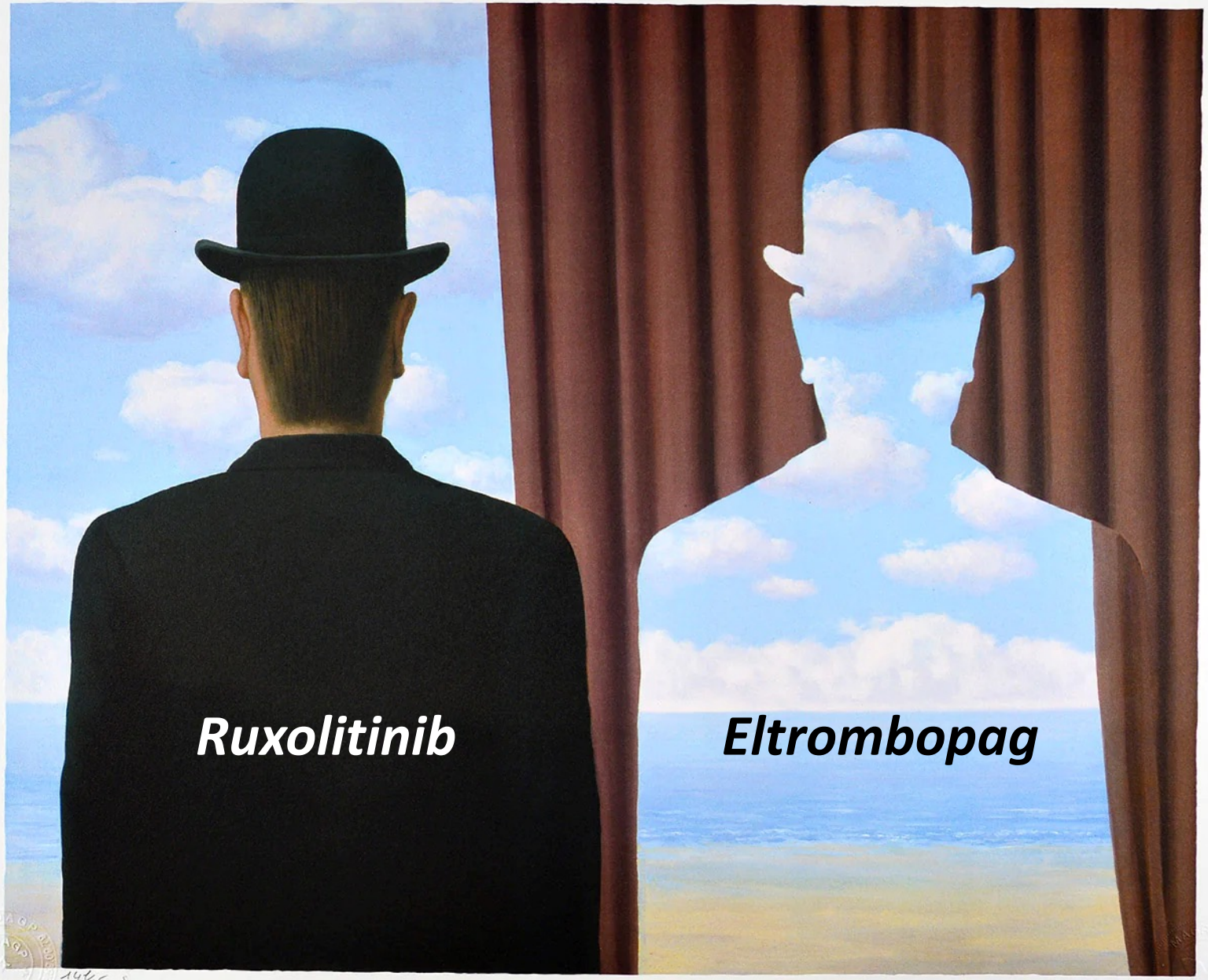
Putative gene network interactions with the dysregulated genes in CD8+ T cells from Ruxolitinib-treated BMF mice





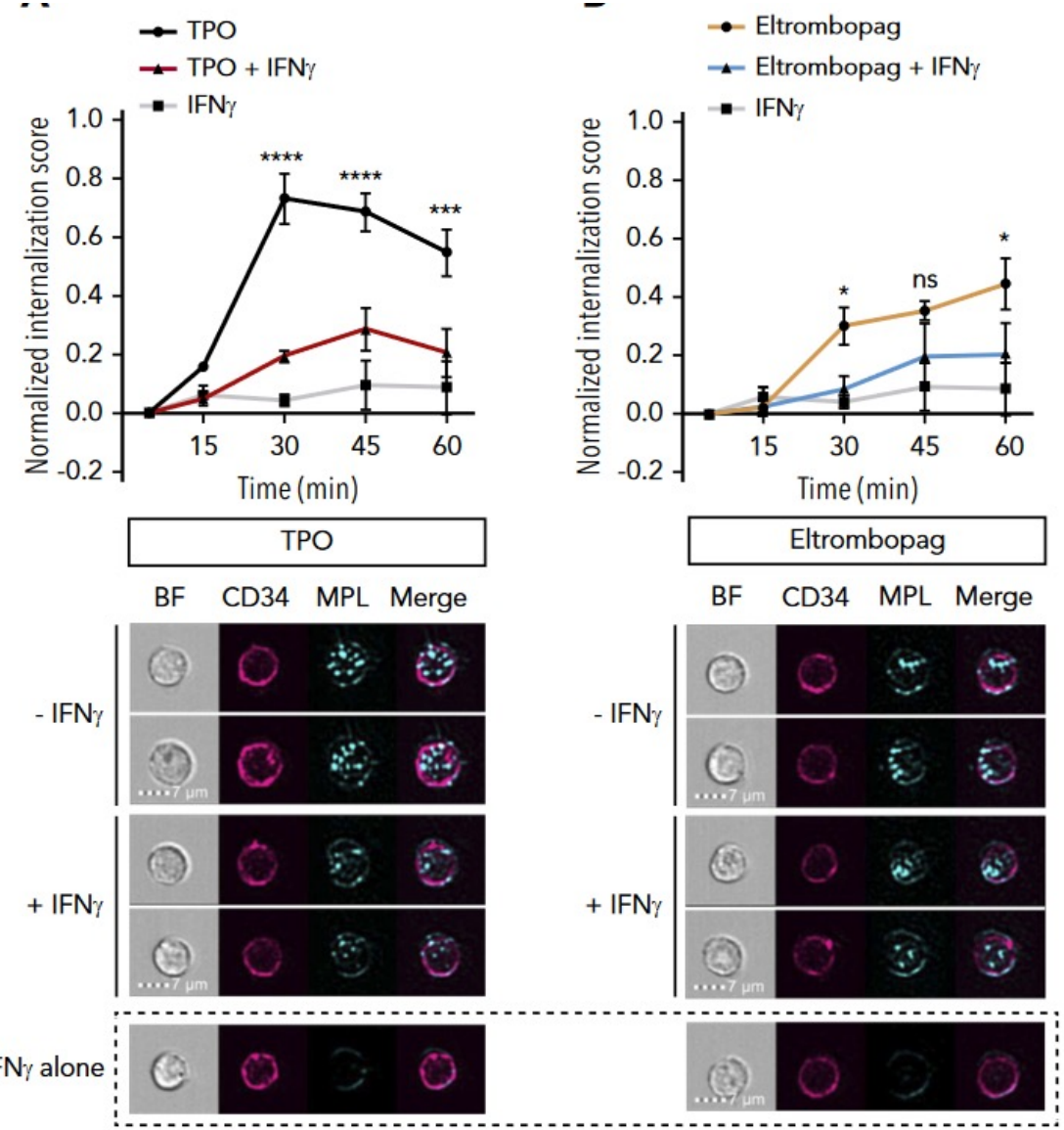
IFNG, STATs, SOCS1

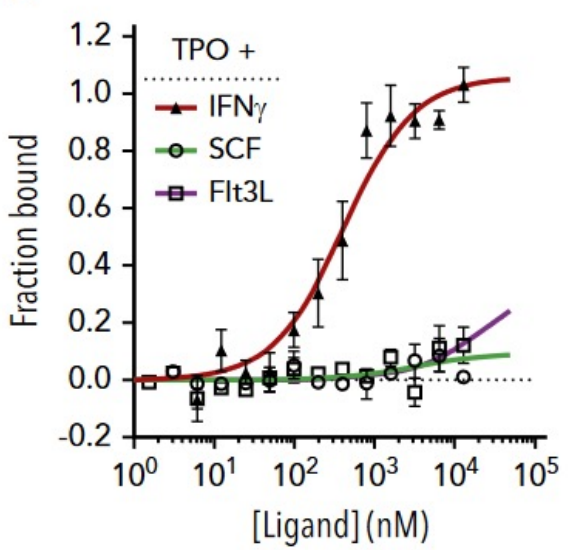
Groarke EM, et al. Blood. 2023 Jan 5;141(1):72-89.

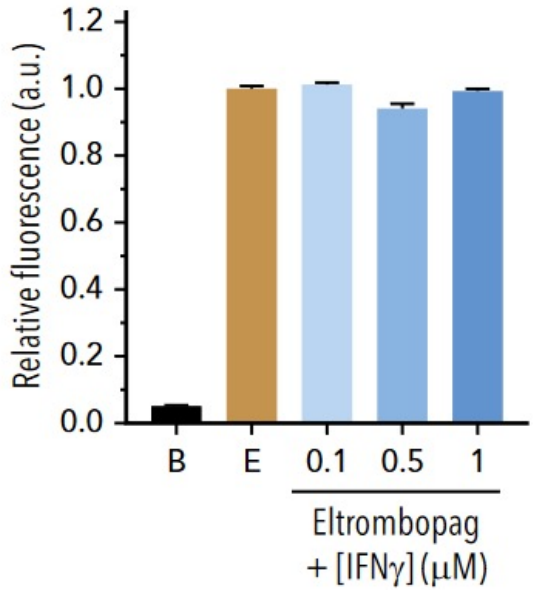


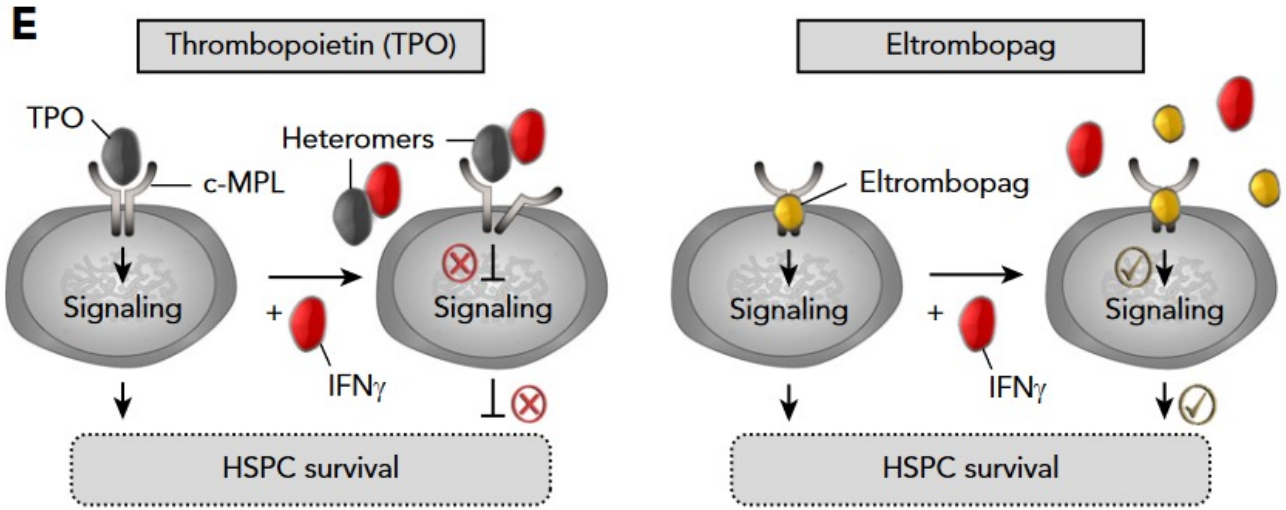
R. Magritte, 1966, Décalcomanie

Targeting IFN-γ pathways – Eltrombopag



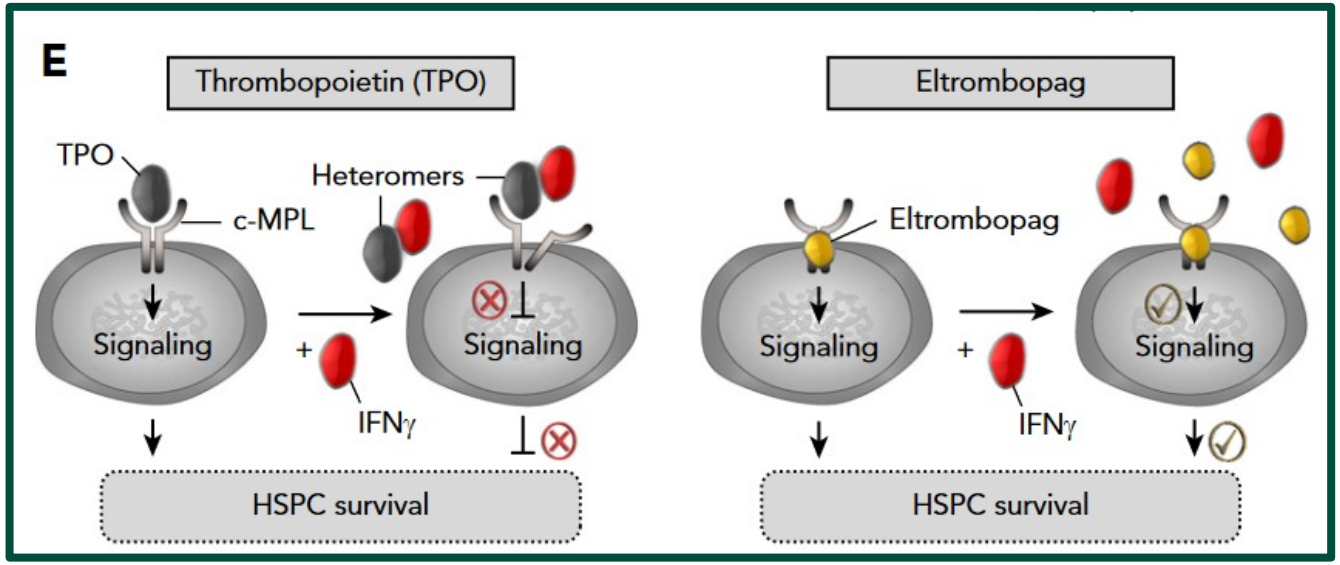


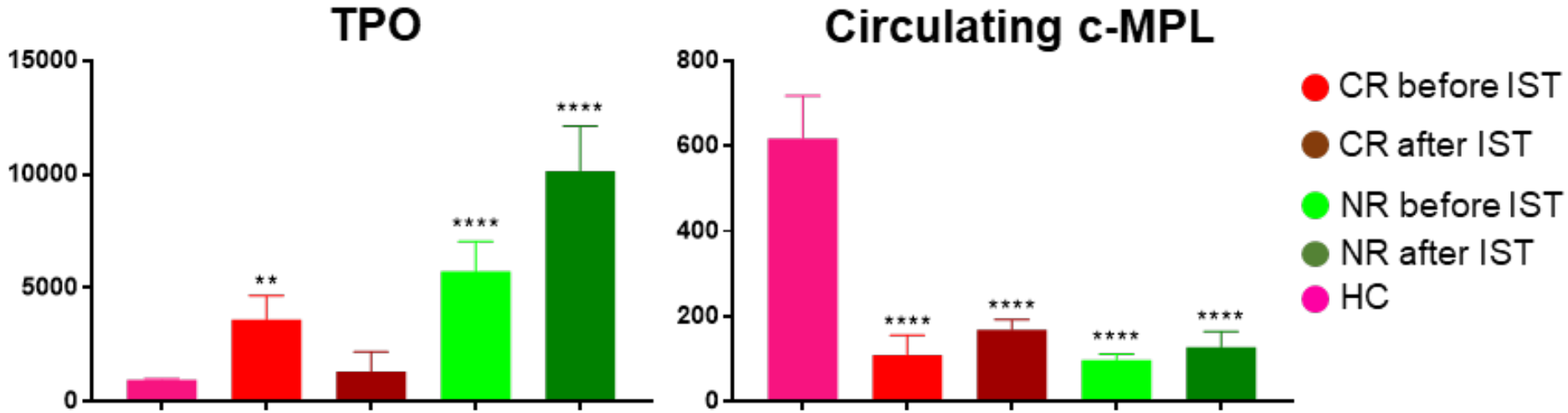




Alvarado LJ, et al. Blood. 2019 May 9;133(19):2043-2055.

Targeting IFN-γ pathways – TPO

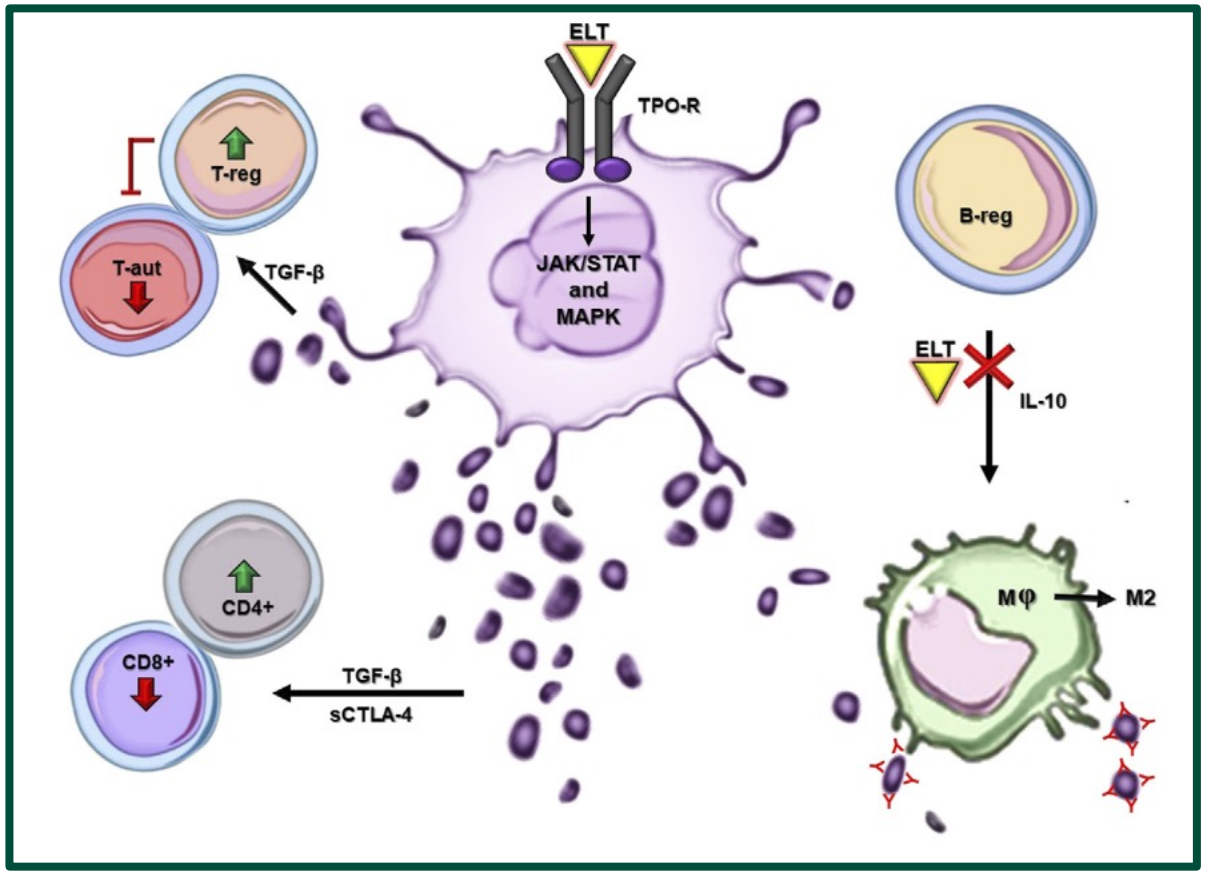




Alvarado LJ, et al. Blood. 2019 May 9;133(19):2043-2055.

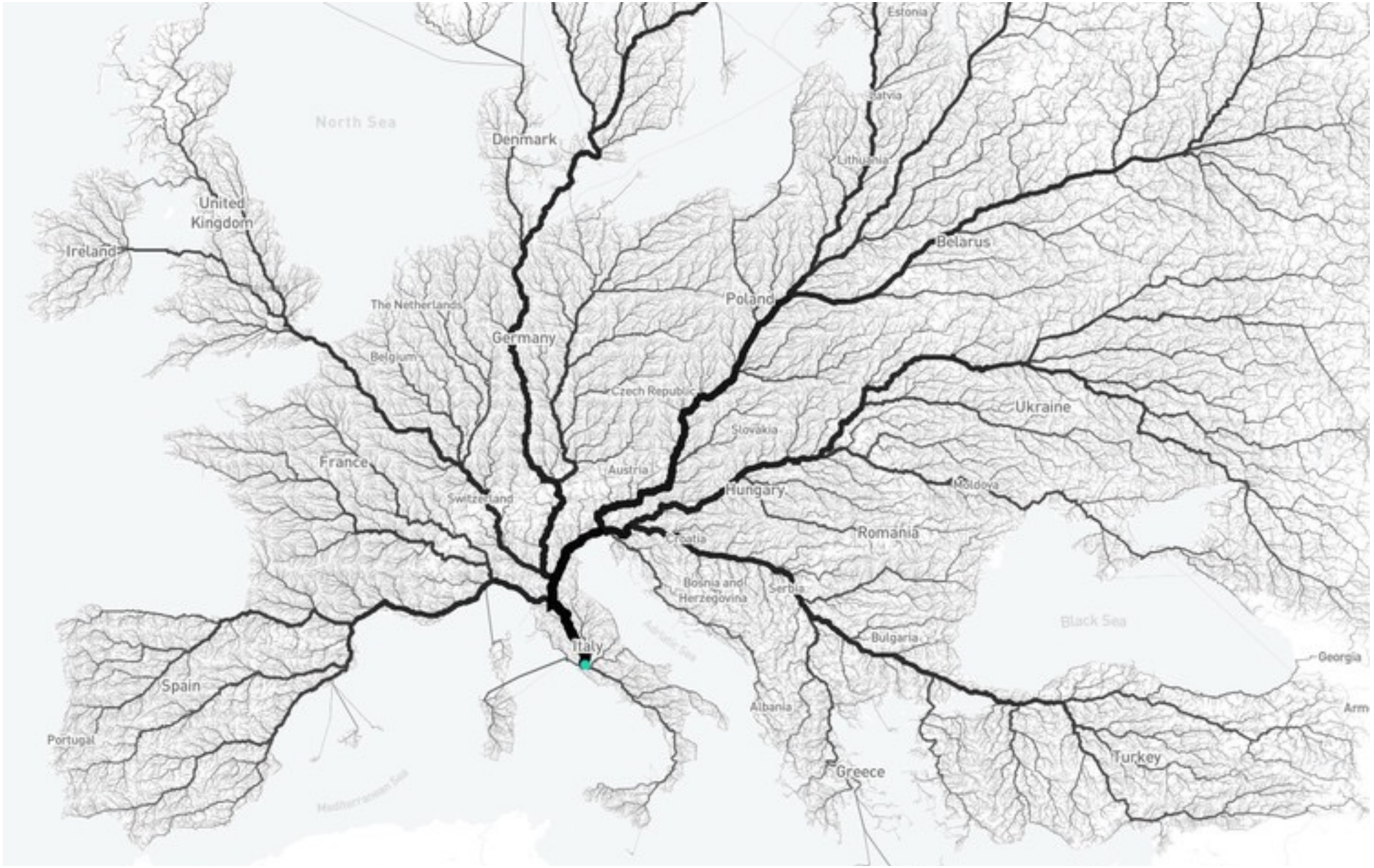
Giudice V, et al. Exp Hematol. 2018 Dec;68:38-50.

Targeting IFN-γ pathways – Growth factors



Disease	ELT effects
SAA	• bi- or tri- lineage hemopoiesis induction • HSCs survival promotion (removing INF-γ inhibition on the TPO-TPO-R axis) • HSCs fitness improvement (through iron chelation) • recovery of BM fitness (through serum ferritin levels reduction) • T helper cells increase and T effector cells reduction as in ITP?
MDS and AML	• megakaryopoiesis improvement • antileukemic effects related to iron chelation • pro-apoptotic signaling activation through ROS modulation • cellular differentiation induction • clonal evolution induction as in SAA?
PGF	• impaired hematopoiesis rescue • human CMV replication inhibition • recovery of BM fitness (through serum ferritin levels reduction) • possible use in GvHD without affecting GvL?

Tarantini F, et al. Front Pharmacol. 2022 May 23;13:906036.



ALL ROADS LEAD TO ROME

Conclusions and future perspectives (1)

- Acquired BMF syndromes are considered immune-mediated disorders because hematological recovery after immunosuppressive therapies is the strongest indirect evidence of the involvement of immune cells in marrow failure development.
- Predominant role of CTLs in marrow destruction, and type I interferons polarizing the immune system toward Th1 responses; however, other T cell subsets are involved.
- IFN-γ and TNF-α are historically implicated in AA pathogenesis.
- Exogenous and stromal cell-produced IFN-γ inhibits HSPC growth and reduces self-renewal of HSCs probably impairing TPO signaling pathways. In addition, IFN-γ directly suppresses erythropoiesis by blocking HPSCs at the earliest stages of differentiation.

Conclusions and future perspectives (2)

- Currently used immunosuppressive therapies might exert their clinical efficacy by directly blocking T cell differentiation and by indirectly interfering with type I IFN responses.
- TPO receptor agonist eltrombopag efficacy might be related to: (i) a direct HSC growth stimulation; (ii) indirect immunomodulatory effects; and (iii) a decoy IFN receptor function.
- JAK1/2 inhibitors are promising therapeutic approach for AA mainly because of their immunomodulatory and anti-inflammatory effects by modulating pro-inflammatory cytokine production.
- **IFN-γ still remains the central cytokine driver of acquired BMF syndromes.**

San Giuseppe Moscati - Avellino
Azienda Ospedaliera di Rilievo Nazionale e di Alta Specializzazione

1° SIMPOSIO SULLE TERAPIE INNOVATIVE IN EMATOLOGIA

Avellino, Hotel de la Ville
30-31 Marzo 2023

GRAZIE PER L'ATTENZIONE

Gli uomini passano, le idee restano.

Restano le loro tensioni morali e continueranno a camminare sulle gambe di altri uomini.

Giovanni Falcone